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A phase II trial of thalidomide in patients with refractory endometrial cancer and correlation with angiogenesis biomarkers: A Gynecologic Oncology Group study

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Abstract

Objectives. A phase II trial was conducted to evaluate the anti-tumor activity and adverse effects of thalidomide in persistent or recurrent endometrial cancer refractory to cytotoxic chemotherapy and to correlate angiogenesis biomarker expression with clinical outcome.

Methods. Consenting patients were treated until progression or intolerable toxicity with an oral starting dose of 200 mg thalidomide/day that was to increase by 200 mg every 2 weeks to a target dose of 1000 mg/day. Vascular endothelial growth factor (VEGF), basic fibroblastic growth factor (bFGF), and soluble endothelial protein C receptor (sEPCR) were analyzed by ELISA in pre and post-treatment specimens.

Results. Twenty-four of twenty-seven patients enrolled in the study were eligible, of whom 2 reached the target dose, 8 progressed before achieving the target dose, and 14 refused or had toxicity that prohibited escalation. Two patients (8.3%) remained progression-free $\geq$6 months. There were 3 (12.5%) with partial responses, 2 (8.3%) with stable disease, 15 (62.5%) with increasing disease, and 4 (16.7%) who were inevaluable for response. Median progression-free survival and overall survival were 1.7 months and 6.3 months, respectively. No grade 4 toxicities were observed. Common grade 3 toxicities included hematologic (n=3), cardiovascular (n=3), constitutional (n=3), and neurologic (n=4). Thalidomide did not decrease VEGF or bFGF levels but reduced sEPCR levels in serum. Elevated plasma vascular endothelial growth factor levels were associated with increased risk of progression and death.

Conclusions. Thalidomide demonstrated limited ability to delay progression (as measured by PFS at 6 months), produce objective responses, or reduce angiogenic marker levels in chemotherapy refractory endometrial cancer. VEGF level appears to be prognostically significant in such patients, independent of thalidomide treatment.

Keywords: Thalidomide; GOG; Refractory endometrial cancer angiogenesis biomarkers

Introduction

Most patients with endometrial cancer will be diagnosed with early stage, favorable prognosis disease, but approximately 7350 patients will die of their cancers each year in the U.S. [1]. For patients with recurrent disease, chemotherapy or progestational agents have been used with palliative intent. Cytotoxic agents including doxorubicin, cisplatin, carboplatin, and paclitaxel have demonstrated activity in phase II studies and have been studied alone or in combination in several phase III trials. In patients with measurable, advanced, or recurrent

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disease, response rates of 27–54% have been reported [2–4]. For patients with advanced stage disease, chemotherapy has been increasingly integrated into first-line therapy. In a randomized trial conducted by the Gynecologic Oncology Group (GOG), doxorubicin and cisplatin chemotherapy produced superior progression-free survival (PFS) and overall survival (OS) compared to whole abdominal irradiation (WAI) [5]. Despite improvements in chemotherapy, the median duration of response for patients with recurrent disease is only 4–8 months, and median survival is generally less than 12 months. As a result, there is continued interest in identifying active agents in cases refractory to cytotoxic drugs.

Recognizing the need to evaluate targeted therapies in patients with endometrial cancer, the GOG introduced a new study queue in 2001 to test novel agents in patients with persistent or recurrent disease who were refractory to cytotoxic chemotherapy. It was hoped that cytostatic targeted agents might prolong PFS in previously treated patient populations by delaying tumor progression but not necessarily producing a significant tumor response. In this setting, such an agent might hold promise for further investigation.

With increased understanding of endometrial cancer at a molecular and genetic level, there is growing interest in identifying targeted biologic agents which affect critical pathways associated with cell growth and death. Angiogenesis has been shown to play an important role in the growth and metastatic potential of many tumors, including endometrial cancer. Attempts to quantify tumor angiogenesis by measuring tumor microvessel density (MVD) or biomarker levels have been made in several studies. In endometrial cancer, higher tumor MVD levels were associated with nodal metastases, advanced stage, and poorer survival [6,7]. Lee and colleagues demonstrated a relationship between vascular endothelial growth factor (VEGF) and MVD levels and found higher tumor VEGF levels to be associated with higher grade tumors, deep myometrial invasion, nodal metastases, and more advanced stage disease [8].

Given the association of angiogenic markers with advanced stage endometrial cancer, an anti-angiogenic agent, thalidomide, was selected for evaluation. The exact mechanism of inhibition of angiogenesis by thalidomide is unknown. Its anti-angiogenic effect is thought to explain the teratogenic effect on limb development during embryogenesis [9]. Thalidomide was approved in 1998 by the FDA for the treatment of erythema nodosum leprosum, and it is currently being tested as an anti-angiogenic therapy for solid tumors in more than 160 clinical trials [10]. It has become particularly important in the treatment of advanced multiple myeloma and is now considered standard in the management of this disease [11]. Thalidomide has been investigated in the treatment of gynecologic malignancies including recurrent ovarian and primary peritoneal cancer, and a 37% response rate was reported in one small phase II trial in patients with chemo-refractory disease [12].

The spectrum of biological activity of thalidomide includes inhibition of angiogenesis, as well as anti-proliferative, pro-apoptotic, and immunomodulatory properties. In some tumor models, thalidomide appears to inhibit expression of pro-angiogenic factors, VEGF and basic fibroblast growth factor (bFGF) [13]. The levels of these biomarkers in tumor tissue and in the circulation are associated with prognosis in multiple cancer types, indicating their utility as surrogates for angiogenesis and suggesting response to anti-angiogenic therapy. Thalidomide has potent anti-inflammatory activity by virtue of its ability to inhibit generation of pro-inflammatory cytokines, down-regulate granulocyte activity, and stimulate cytotoxic Th1 T cell proliferative responses with ensuing production of interferon γ [14]. Modulation of an inflammatory environment is reflected in changes in the soluble endothelial protein C receptor (sEPCR) biomarker, a member of the protein C anti-coagulant and anti-inflammatory pathway [15,16]. Changes in these biomarkers may reflect either selective cancer regression or more systemic responses as a result of therapy. Taking into consideration thalidomide's diverse activities, acceptable toxicity profile, convenience of oral dosing, and activity seen in other disease sites, it was determined to be an interesting and appropriate agent for study in patients with recurrent endometrial cancer.

The primary objectives of this study were to estimate the anti-tumor cytostatic activity of thalidomide as measured by the proportion of patients alive and progression-free at 6 months and to determine the nature and degree of toxicity conferred by thalidomide. Secondary objectives were to determine the PFS and OS, as well as the frequency of complete and partial responses, in this patient population. The associated translational research objectives were to determine the effects of thalidomide on VEGF, bFGF, and sEPCR levels in serum and plasma and to explore the association of these biomarkers with clinical outcome.

Materials and methods

Study population

Women with histologically confirmed persistent or recurrent endometrial cancer of any subtype were eligible. Pathology from the primary tumor was centrally reviewed. Patients could have received one prior chemotherapy regimen if their GOG performance status (PS) was 0–2 or two prior regimens if they had a PS of 0–1. All patients were required to have measurable disease using the RECIST criteria and acceptable baseline laboratory values including absolute neutrophil count >1500/μl, platelet count >100,000/μl, creatinine <1.5 times institutional upper limit of normal, bilirubin <1.5 times institutional upper limit of normal, and SGOT and alkaline phosphatase <2.5 times institutional upper limit of normal. Preexisting neuropathy (sensory and motor) had to be ≤ grade 1. The protocol contained provisions for the exclusion and prevention of pregnancy; however, all patients were post-menopausal or had prior hysterectomy. Patients were ineligible if they had received prior thalidomide, had brain metastases, or were receiving bisphosphonates. Prophylactic use of anti-coagulation (warfarin, low-molecular weight heparin) was not permitted. Patients provided written informed consent consistent with federal, state, and local institutional requirements to participate in the clinical and translational research components of this protocol prior to receiving any protocol therapy. In addition, the protocol was approved by the GOG, the Cancer Therapy Evaluation Program of the National Cancer Institute, and the institutional review board at each of the participating GOG institutions and was performed in accordance with assurances filed with and approved by the Department of Health and Human Services.

Treatment plan and outcome measurements

Eligible patients initiated therapy with a daily oral dose of 200 mg thalidomide. Each 28-day period constituted one treatment cycle. Patients were instructed to maintain a dosing and toxicity log which was reviewed by study nurses every 2 weeks. For patients not experiencing grade 3–4 toxicities,

thalidomide was to be increased by 200 mg at approximately two-week intervals until a target dose of 1000 mg per day was reached. Patients who did not tolerate the higher dose maintained the highest tolerable dose level. Dose escalations were slowly titrated due to the anticipated tachyphylaxis to sedation and somnolence. While on treatment, patients were to be examined monthly and were assessed for disease response or progression using RECIST criteria every other cycle [17]. Toxicity was to be assessed after every cycle of therapy and was scored based on the NCI Common Toxicity Criteria, version 2 (CTCv2). Patients were to remain on study drug at the highest tolerable dose until evidence of disease progression or development of unacceptable toxicity.

Specimen collection procedures

Serum and plasma were obtained from all patients at two time points as of October 12, 2001. Pretreatment specimens were collected within 14 days prior to starting cycle 1. Pre-cycle 2 specimens were collected within 4–6 weeks after starting cycle 1 and just before starting cycle 2 of treatment, or when protocol therapy was discontinued during cycle 1 due to excessive toxicity or disease progression. Serum was prepared from 10 ml of blood drawn into a serum separator tube, aliquoted, and frozen in an ultra-cold freezer (≤ −70 °C) or a non-cycling −20 °C freezer. A platelet count was obtained at each time point in an effort to adjust for the potential contribution of VEGF that may arise from platelets that lyse during the serum preparation procedure [18]. Plasma was prepared from blood drawn into a lavender blood collection tube with the anticoagulant EDTA, aliquoted, and frozen. Frozen pre-cycle 1 and pre-cycle 2 specimens (serum and plasma) were shipped to the GOG Tissue Bank (Columbus, Ohio) with dry ice within 4 weeks or 10 weeks of the start of treatment, respectively. Batches of frozen specimens from patients participating in this protocol were then shipped to the University of Oklahoma Health Science Center for testing.

Quantification of VEGF, bFGF, and sEPCR

An enzyme-linked immunosorbent assay (ELISA) was used to quantify the concentration of VEGF, bFGF, or sEPCR. All specimens were assayed for an individual biomarker on the same day. Standards (VEGF: 32.2 to 1000 pg/ml, bFGF: 10 to 320 pg/ml, or sEPCR: 1.47 to 23.53 ng/ml) and controls were included on each ELISA plate. Paired specimens (pre-cycle 1 and pre-cycle 2 serum and plasma) from a particular patient were evaluated on the same ELISA plate. Individual specimens were evaluated in duplicate, thawed just prior to the assay, and randomly positioned on each ELISA plate. Validated ELISA kits from R&D Systems, Inc (Minneapolis, MN) were used to quantify the concentration of VEGF (Quantikine Human VEGF Immunoassay-Catalog SVE00) or bFGF (Quantikine Human bFGF Immunoassay-Catalog SSFB75) in undiluted serum and plasma specimens as described by the manufacturer. Absorbance was measured using an automatic microplate reader at 450 nm and 540 nm. The concentration of VEGF or bFGF in each sample was interpolated from the plate-specific standard curve after subtracting the background staining at 540 nm from the absorbance measured at 450 nm. sEPCR concentration was quantified in serum and plasma using a commercially available ELISA kit (Asserachrom® sEPCR, Diagnostica Stago, Asnières, France). The concentration of sEPCR in each sample was interpolated from the plate-specific standard curve from absorbance measured at 450 nm. ELISAs were performed blinded to any clinical information and laboratory data connected with specimen identifiers rather than case identifiers were transferred to the GOG Statistical and Data Center for analysis.

Statistical design and methods

A two-stage design was used to evaluate the activity of thalidomide using nearly optimal but flexible decision rules [19] based on the number of patients who survived progression-free for at least 6 months. The targeted sample size for the first stage of accrual was 25 eligible and evaluable patients, but in practice the accrual size was permitted to range from 22 to 29 patients for administrative reasons. If there were more than 3 out of 22–24, or more than 4 out of 25–29, patients alive and progression-free for at least 6 months and medical judgment so indicated, accrual to the second stage of the trial was to be initiated. Otherwise, accrual was stopped as the treatment regimen was deemed to be not clinically promising. If the study advanced to the second stage, an overall study accrual of 56 eligible and evaluable patients would be targeted, but the permissible range was from 53 to 60 patients, for administrative reasons. If no more than 10 out of 53, 11 out of 54–57, or 12 out of 58–60 patients were alive and progression-free after 6 months, the regimen would be considered clinically uninteresting. If the true probability of observing a 6-month PFS was 15%, the decision rules limited the probability of designating the treatment as active to 10% with an average probability of early termination after the first stage equal to 59%. On the other hand, if the true probability of having a 6-month PFS was 30%, the average probability of correctly classifying the treatment as active was 90%. PFS was calculated from study entry until disease progression, death, or date of last contact. The frequency of response is defined as the sum of complete and partial responses using RECIST criteria. Exploratory data analyses were performed to determine effects of thalidomide on VEGF, bFGF, and sEPCR in serum and plasma and to assess the association of these biomarkers with clinical outcome. Statistical analyses were performed using SAS for Windows, version 9.1 software (SAS Institute, Inc. Cary, NC).

Results

From August of 2001 to July of 2002, 27 patients with chemo-refractory endometrial cancer were enrolled in this study. Of these, three patients were ineligible: 1 endometrial cancer was a second primary, 1 had received more than 2 prior therapies, and 1 had no prior chemotherapy (cisplatin was given as a radiosensitizer). The median age of the 24 eligible patients was 70 years. Pretreatment characteristics are shown in Table 1.

Table 1
Patient characteristics ($n=24$)

Age (years)		
Median	70	
Range	51–85	
	No.	%
Race		
Asian	1	4
Black	1	4
White	22	92
GOG performance status		
0	12	50
1	10	42
2	2	8
Cell type		
Endometrioid	9	38
Serous	7	29
Mixed	4	17
Adenosquamous/squamous	2	8
Clear cell	1	4
Undifferentiated	1	4
Grade		
Well differentiated	4	17
Moderately differentiated	5	21
Poorly differentiated	15	62
Prior chemotherapy		
1 regimen	14	58
2 regimens	10	42
Prior radiation therapy		
Yes	15	62
No	9	37
Prior hysterectomy		
Yes	23	96
No	1	4

Table 2
Treatment outcomes ($n=24$)

Outcome	No.	%
Progression-free survival		
≥6 months	2	8
<6 months	22	92
Response		
Complete	0	0
Partial	3	12
Stable disease	2	8
Progression	15	63
Not evaluable	4	17
Number of cycles received		
1	10	42
2	9	38
3	1	4
4	3	12
9	1	4

Table 3
Adverse events ($n=24$)

	Grade	
	2	3
Anemia	5	0
Hematologic	0	3
Cardiovascular	1	3
Coagulation	0	1
Constitutional	5	3
Dermatologic	1	0
Gastrointestinal	10	2
Genitourinary/Renal	0	0
Hemorrhage	1	0
Hepatic	1	0
Lymphatics	0	0
Musculoskeletal	2	1
Metabolic	0	0
Neuropathy — motor	1	1
Neuropathy — sensory	3	1
Depressed level of consciousness	4	1
Other neurologic	2	2
Pain	4	0
Pulmonary	4	1

There were no grade 4 toxicities reported.

Most patients (62.5%) had received prior radiation therapy, all had been treated with at least one and 41.7% had received two prior chemotherapy regimens. The most frequent tumor histology was classified as endometrioid (9 cases, 38%), serous (7 cases, 29%), and mixed (4 cases, 17%). All patients initiated treatment with thalidomide at 200 mg/day, but few were able to escalate their doses due to disease progression or toxicity. Of the 24 patients, 16 (66.7%) were treated at the first two dose levels (200 mg/day [$n=9$] or 400 mg/day [$n=7$]). There were an additional three patients each treated with a maximum dose of either 600 or 800 mg/day. Only two patients received the target dose of 1000 mg/day of thalidomide. Eight patients had disease progression during drug escalation, thirteen had toxicity that prevented escalation, and three patients refused escalation. Although there were no complete responses observed in this study, three patients (12%) experienced a partial response (90% confidence interval [CI]: 3.5–29.2%) and two (8%) had stable disease. Two patients (8%) (90% CI: 1.5–24.0%) maintained a PFS ≥6 months (Table 2). The median PFS was 1.7 months and median duration of survival was 6.3 months (Fig. 1). No grade 4 toxicities were observed. The number of patients experiencing grade 3 toxicities is shown in Table 3, and most frequently included hematologic, cardiovascular, constitutional, and neurological toxicities.

VEGF, bFGF, and sEPCR levels were quantified in serum and plasma prepared from blood drawn prior to starting cycles 1 and 2 of thalidomide treatment. Analyses revealed that serum

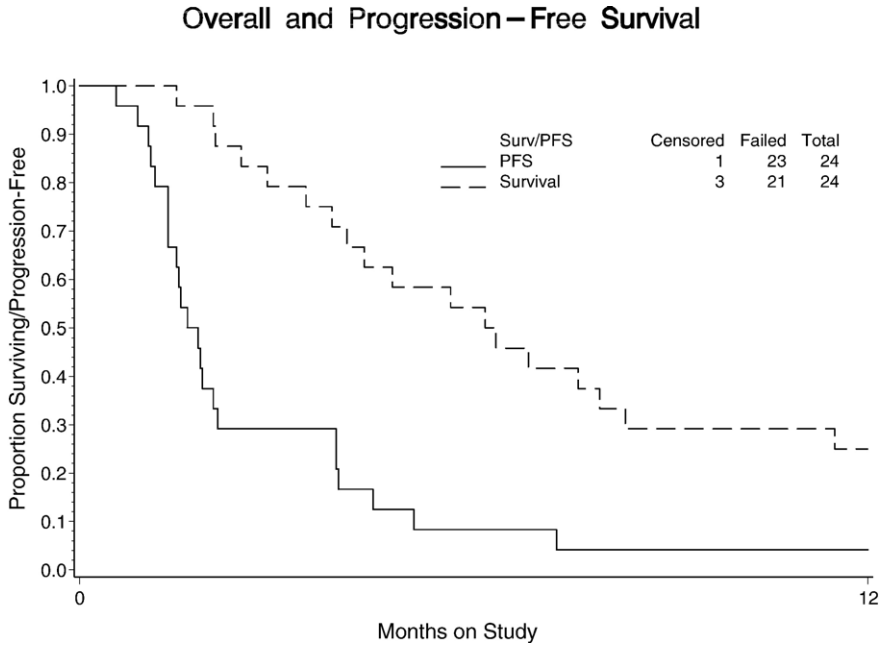

Fig. 1. Progression-free survival and overall survival (Kaplan–Meier).

concentrations of VEGF were significantly higher than plasma concentrations, but VEGF concentrations in serum compared with plasma were not strongly correlated. Both plasma and serum levels were determined in an effort to account for the possibility of release of VEGF from platelets during clotting to prepare the serum. After adjusting for the influence of one observation, platelet count prior to starting cycle 1 or cycle 2 of therapy was not significantly associated with the pre-cycle 1 or pre-cycle 2 serum concentration of VEGF. It also did not account for the observed variability in the concentration of VEGF between serum and plasma (Table 4).

Given that the sample size in this study was small and data appeared to be non-normal for some of the biomarkers, a Wilcoxon signed rank test was used to evaluate changes in biomarker concentrations prior to starting cycles 1 and 2. Thalidomide did not appear to decrease the level of VEGF or bFGF in serum or plasma. In contrast, a decrease in serum level of sEPCR corresponded with exposure to thalidomide ($p=0.018$). It was not possible to evaluate changes in sEPCR in pre-cycle 1 and pre-cycle 2 plasma specimens given that only two matching observations were available for analysis.

Table 5
Associations of biomarkers with clinical outcome[a]

		PFS			OS		
		HR[b]	95% CI	*p*-value	HR b	95% CI	*p*-value
VEGF	Plasma (PB01)	3.5	1.5–7.8		4.1	1.5–11.3	0.006
	Serum (SB01)	1.1	0.6–1.8	0.810	0.8	0.5–1.4	0.532
bFGF	Plasma (PB01)	0.9	0.5–1.5	0.620	0.7	0.3–1.5	0.370
	Serum (SB01)	1.8	0.9–3.4	0.077	3.5	0.3–36.7	0.299
sEPCR	Plasma (PB01)	0.7	0.3–1.9	0.531	0.5	0.2–1.4	0.185
	Serum (SB01)	1.0	0.6–1.7	0.929	1.0	0.6–1.6	0.938
	Serum difference (SB02-SB01)	1.4	0.6–3.4	0.476	1.0	0.5–1.9	0.892

HR: hazard ratio; 95% CI: 95% confidence interval.
[a] Cox regression analysis adjusting for histologic subtype and GOG performance status.
[b] For one standard deviation increase in concentration while holding histologic subtype and performance status constant. Units for VEGF and bFGF are in pg/ml; sEPCR is in ng/ml.

Cox proportional hazard modeling was used to evaluate whether or not any of these serum and plasma biomarkers predicted PFS and OS (Table 5). Pretreatment VEGF level in plasma (not serum) was associated with an increased risk of

Table 4
Laboratory results and clinical outcomes

Maximum dose (mg)	Cell type	VEGF (pg/ml)				bFGF (pg/ml)				sEPCR (ng/ml)				PFS ≥6 months	Tumor response
		Plasma		Serum		Plasma		Serum		Plasma		Serum[a]			
		Pre-cycle 1	Pre-cycle 2	Pre-cycle 1	Pre-cycle 2	Pre-cycle 1	Pre-cycle 2	Pre-cycle 1	Pre-cycle 2	Pre-cycle 1	Pre-cycle 2	Pre-cycle 1	Pre-cycle 2		
200	E	N/A	16.1	1407.0	1412.8	N/A	4.6	4.9	6.1	N/A	2.4	2.4	1.9	No	ID
	S	884.8	N/A	1243.1	N/A	32.0	N/A	4.6	N/A	2.4	N/A	N/A	N/A	No	ID
	S	53.7	45.4	N/A	171.8	7.4	6.6	N/A	7.0	2.5	2.1	N/A	2.0	No	ID
	E	30.8	405.8	605.4	701.0	5.5	9.4	5.3	7.0	1.8	N/A	2.1	1.8	No	SD
	E	201.4	155.4	1078.8	1121.6	4.8	5.6	4.5	4.5	2.4	2.6	2.2	2.8	No	N/E
	E	11.3	9.6	187.8	199.4	4.6	4.1	4.3	4.2	N/A	N/A	3.4	3.4	No	N/E
	U	N/A	N/A	770.9	N/A	N/A	N/A	5.5	N/A	N/A	N/A	2.7	N/A	No	ID
	E	N/A	48.4	300.7	438.5	N/A	7.6	5.3	5.4	N/A	5.1	6.9	5.3	No	ID
	E	N/A	915.0	1075.5	N/A	N/A	N/A	4.7	N/A	N/A	N/A	2.4	N/A	No	ID
400	S	509.3	N/A	395.0	N/A	N/A	N/A	4.1	N/A	N/A	N/A	2.8	N/A	No	ID
	M	N/A	N/A	754.9	N/A	N/A	N/A	4.3	N/A	N/A	N/A	2.7	N/A	No	N/E
	S	183.1	N/A	566.4	N/A	4.5	N/A	4.5	N/A	2.5	N/A	2.9	N/A	No	ID
	E	1418.5	8.9	995.1	8.2	6.5	4.5	139.1	4.2	2.4	N/A	2.5	N/A	No	ID
	E	239.7	321.4	117.7	9.8	5.7	4.2	N/A	4.7	3.9	N/A	N/A	N/A	No	ID
	S	N/A	94.0	439.5	653.6	N/A	6.1	5.2	4.9	N/A	5.8	5.4	6.1	No	ID
	S	15.04	N/A	164.4	173.4	5.7	N/A	7.0	5.4	2.1	N/A	2.5	2.0	No	PR
600	M	37.3	64.9	535.1	478.8	7.2	8.5	4.3	4.8	N/A	2.3	3.1	2.6	Yes	PR
	M	26.7	27.4	917.1	940.9	4.5	4.2	4.4	4.6	2.7	N/A	3.2	3.1	No	N/E
	S	80.1	721.8	701.8	911.2	6.6	78.0	8.1	4.9	2.8	N/A	3.0	2.5	No	PR
800	A	N/A	197.2	586.1	816.7	N/A	4.6	5.9	8.7	N/A	0.9	4.6	3.0	No	ID
	Sq	710.9	N/A	N/A	N/A	4.2	4.2	N/A	N/A	1.4	N/A	N/A	N/A	No	ID
	M	17.5	16.2	58.9	150.8	4.8	4.3	7.6	9.6	3.5	N/A	3.5	2.8	No	ID
1000	E	23.1	155.9	647.3	937.4	6.8	44.3	5.1	4.9	2.0	N/A	2.5	1.7	No	ID
	C	375.7	96.3	1106.9	891.4	43.5	4.5	4.9	4.4	N/A	2.1	2.5	1.7	Yes	SD
n		17	17	22	17	16	17	21	17	13	8	20	15		
M		80.1	94.0	626.3	653.6	5.7	4.6	4.9	4.9	124.8	120.8	140.0	132.4		
SD		393.7	262.3	381.2	423.5	11.2	19.5	29.2	1.6	33.1	84.1	61.0	65.6		

E: endometrioid adenocarcinoma; S: serous adenocarcinoma; U: undifferentiated carcinoma; M: mixed epithelial carcinoma; A: adenosquamous carcinoma; Sq: squamous cell carcinoma; C: clear cell carcinoma; N/A: not available because the specimen was not submitted, was received in unsatisfactory condition, or was used up prior to testing; *n*: number of cases with evaluable laboratory data; *M*: median; SD: standard deviation; ID: increasing disease; SD: stable disease; N/E: not evaluable; PR: partial response.
[a] Exact Wilcoxon signed-rank test: $n=14$; statistic=19; $p=0.018$.

progression ($p=0.003$) and death ($p=0.006$) after adjusting for histologic subtype and GOG performance status. Neither plasma or serum bFGF nor sEPCR levels was a predictor of PFS or OS. The difference in the serum concentration of sEPCR was also not a predictor of clinical outcome.

Discussion

Thalidomide as an anticancer agent was first reported in 1965 where it demonstrated minimal activity in 71 patients with advanced malignancies [20]. Of note, one of three endometrial cancer patients demonstrated stable disease for 1 year while on treatment. The exact mechanism of action of thalidomide is unknown; however, data point to inhibition of angiogenesis for its anti-cancer role [9]. Thalidomide has been shown to reduce corneal neovascularization induced by bFGF in rabbit model and by VEGF in a mouse model [21]. It also reduces tumor necrosis factor-alpha (TNF-α) production by monocytes and macrophages. Animal models demonstrated thalidomide activity against solid tumors in rabbits and nude mice, and it produced apoptosis in established tumors [22,23]. Singhal and colleagues were among the first to report significant activity of thalidomide in a population of patients with advanced, previously treated multiple myeloma [11]. Using an escalated dose capped at 800 mg/day, complete response was seen in 10% of patients, and 32% demonstrated a 25% reduction in urine or serum paraprotein levels. Thalidomide has been studied in a variety of solid tumor types including glioma, melanoma, renal cell carcinoma, prostate, breast, and ovarian cancers. The present study using thalidomide represents the GOG's first experience with a targeted biologic agent in endometrial cancer.

Angiogenesis is felt to be an important potential target in endometrial cancer. Increased angiogenicity as measured by MVD counts has been shown in hyperplastic and malignant endometrium compared to normal controls [24]. Microvessel density has also been correlated with increasing disease stage and poorer outcomes. Salverson showed that 5-year survival was 57% versus 90%, respectively, in patients with higher versus lower MVD, and MVD was an independent predictor of survival in a multivariate analysis [6].

Angiogenic biomarkers have also been correlated with endometrial cancer stage and prognosis. A study of 53 patients found significant associations between VEGF expression and disease progression, as well as lymph node metastases [25]. Hirai and colleagues reported on 228 endometrial cancer cases stained for VEGF expression [26]. They found that VEGF expression was associated with depth of invasion, lymphovascular space invasion, and nodal metastases. VEGF status was also an independent predictor of 5- and 10-year survival. In other studies, however, VEGF expression did not correlate with the incidence of metastases, recurrence, and survival [27–29]. The reason for the discrepancies between studies may lie in the varying expression pattern of VEGF in early versus late stage endometrial cancer. VEGF is highly expressed in early stage and well-differentiated uterine endometrial cancers, but expressed at lower levels in advanced clinical stage and moderately or poorly differentiated tumors [30]. The expression of bFGF, however, exhibits the opposite pattern, with low expression in early stage endometrial cancer and overexpression in advanced and poorly differentiated uterine endometrial cancers [30]. A single study of cellular bFGF expression in 66 endometrial carcinomas reported a statistically significant correlation with tumor grade and high MVD [31]. Combined expression of bFGF and high MVD was significantly associated with worse prognosis.

Significant associations have also been shown between VEGF levels and tumor stage, MVD, and tumor VEGF expression in a wide variety of malignancies. Sustained VEGF production by tumor and surrounding stroma is thought to be crucial in establishing the angiogenic process. While immunohistochemical detection of cytoplasmic VEGF can be used to evaluate prognosis in primary endometrial cancer, measurement of circulating VEGF levels is a more feasible approach in the situation of recurrent or persistent endometrial cancer where cancer tissue is not always available. Multiple studies using enzyme-linked immunosorbent assay (ELISA) of serum demonstrated significantly higher levels of circulating VEGF in endometrial cancer patients in comparison to healthy controls [32–34]. In the present study, higher pretreatment VEGF levels in plasma were associated with an increased risk of disease progression and death. This observation confirms previously published results in patients with endometrial cancer [25,26,35] and the findings of two recent phase II studies conducted by GOG in patients with leiomyosarcoma of the uterus and in patients with persistent or recurrent ovarian or primary peritoneal cancers (unpublished results).

It has also been hypothesized that measuring VEGF levels in serum and plasma might reflect efficacy of anti-angiogenic therapies. In one study of 72 patients with endometrial cancer, including 27 with endometrial hyperplasia and 30 healthy controls, circulating VEGF levels were associated with tumor stage [34]. VEGF levels in patients with advanced stage disease decreased significantly after treatment with adjuvant chemotherapy then increased at clinical relapse. Studies have shown modulation of angiogenic biomarkers due to thalidomide and reduction in growth factor levels to be inconsistent. Bertolini showed reductions in VEGF and bFGF levels which corresponded to clinical response in patients with multiple myeloma treated with thalidomide [13]. However, in a phase II study of low dose thalidomide (100 mg/day) to treat 66 patients with advanced renal cell carcinoma, melanoma, ovarian cancer, or breast cancer, no relationship was seen between serum VEGF or bFGF levels and treatment or response [36]. In an effort to better understand the role of angiogenesis in endometrial cancer, serum and plasma levels of VEGF and bFGF were measured prior to and after 4–6 weeks of thalidomide. No significant alterations in VEGF or bFGF levels were observed between pre- and post-treatment samples.

Given that platelets are an important source of circulating VEGF, both plasma and serum levels were monitored in the current study. We found that serum levels were about 7-fold higher than plasma levels, but platelet count did not correlate with serum concentrations of VEGF (R^2 0.14). Although VEGF

and bFGF are key regulators of angiogenesis, the strength of this study is limited by evaluation of only two of the known angiogenic cytokines, while it is known that angiogenesis is modulated by complex interactions of multiple cytokines, sex steroid hormones, hypoxia, and other factors.

An exploratory evaluation of another factor which may respond to thalidomide treatment was also evaluated. The protein C pathway is a primary regulator of coagulation and inflammation events [37]. Protein C is activated on endothelial cells by the concerted activities of thrombin, thrombomodulin, and the endothelial protein C receptor (EPCR). Two recent studies demonstrate increased expression of EPCR on cancer cell lines and in some human tumor cells [38,39]. The significance of these observations is not known with respect to either protein C pathway function or tumor survival and metastasis. A functional, soluble form of EPCR (sEPCR) circulates in plasma which is a proteolytic product of the membrane-bound EPCR. sEPCR levels are elevated in patients with systemic inflammatory diseases and there is evidence that sEPCR levels increase as a result of thrombin activity [40,41]. Many cancers overexpress tissue factor, the membrane receptor that is ultimately responsible for *in vivo* thrombin generation and contributes to tumor invasive potential [42]. Since sEPCR levels reflect thrombin activity, we explored whether sEPCR could be a potential biomarker of treatment response and prognosis. In this study, sEPCR was the only biomarker studied which showed a statistically significant reduction following treatment with thalidomide. The actual significance of this effect remains uncertain as reduction in sEPCR levels was not associated with clinical benefit. Further work is ongoing to study circulating sEPCR levels in patients with gynecologic malignancies to evaluate whether changes in sEPCR levels may be associated with prognosis or response to therapy. To our knowledge, there are no prior studies of sEPCR levels in cancer patients.

It was postulated that cytostatic agents such as thalidomide might more likely delay tumor progression than produce objective response. As such, the primary outcome measure used in this study to determine efficacy was different than has been used in GOG phase II studies evaluating a cytotoxic agent. Only 8% of patients in this study experienced a delay of tumor progression of >6 months. By comparison, our experience with cytotoxic agents in similar populations showed that approximately 20% of patients remain progression-free at 6 months (unpublished data). Likewise, response rates, and median PFS and OS, were lower with thalidomide than had been reported with previously tested cytotoxic agents [43,44]. The minimal activity of thalidomide in this setting could be due to advanced disease stage, which may not allow sufficient time for angiogenic therapy to take effect. Tumor regression by anti-angiogenic therapy can be slow, taking more than a year [45]. Advanced disease stage could also be responsible for greater genetic instability of the tumor cells, allowing tumors to become resistant to the anti-angiogenic effects of thalidomide [10]. Thus, combinations of molecularly targeted anti-angiogenic compounds that block different points in the pathway may be needed in advanced disease to avoid development of resistance (or early combination of chemotherapy and an anti-angiogenic agent).

The toxicities of thalidomide are dose-dependent. Constipation, somnolence, rash, and a potentially irreversible peripheral neuropathy have been most commonly reported. Less frequently, seizures, predominantly in patients with CNS metastases, and deep venous thrombosis have also been reported. Singhal found that the frequencies of toxicities were dose-dependent over the range of 200–800 mg/day [11]. At doses of 200 mg/day, paresthesia of extremities was present in 12% of patients and rose to 28% of patients at 800 mg/day. Of note, less than 10% of the 84 patients experienced any grade 3–4 toxicity. In the present study, no grade 4 toxicities were seen, and grade 3 toxicities were infrequent and managed by dose reduction. Because somnolence and sedation are common and improve over time, therapy was initiated at 200 mg/day and escalated every 2 weeks. In this patient population, it was difficult to reach the target dose of 1000 mg/day due to disease progression. Results must be viewed in the context that only two patients in this study were able to reach the target dose of 1000 mg/day and therefore reflect the assessment of toxicity at predominantly low dose levels (200–400 mg/day).

In conclusion, thalidomide did not demonstrate substantial activity as judged by the ability to prevent or delay disease progression for at least 6 months in this patient population. The fact that many patients progressed before the target dose could be reached speaks in part to the rate of tumor growth, the extent of disease at study enrollment, and the difficulty of using an agent which requires slow titration. Thalidomide also demonstrated only limited ability to produce objective responses or reduce angiogenic marker levels in persistent or recurrent EC. Although the concentration of sEPCR significantly decreased with time (corresponding with thalidomide exposure), the reduction in the level of sEPCR was not associated with clinical outcome. The pretreatment level of VEGF in plasma was associated with an increased risk of disease progression and death. Despite our findings, we suspect that angiogenesis is an important process to target in endometrial cancer, and the GOG will evaluate other anti-angiogenic agents in the future.

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