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Evaluation of Potentially Predictive Markers for Anti-Angiogenic Therapy with Sunitinib in Recurrent Ovarian Cancer Patients[1]

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Abstract

INTRODUCTION: In ovarian cancer, new therapeutic strategies are needed because the vast majority of patients develop a recurrence and resistance to platinum derivates. Attached to the AGO-OVAR2.11 study investigating the multityrosine kinase inhibitor sunitinib in recurrent platinum refractory ovarian cancers, this translational research project assesses the potential value of serum vascular endothelial growth factor (VEGF), soluble VEGF receptor-3 (sVEGFR-3), and angiopoietin-2 (Ang-2) levels for progression-free survival (PFS). *MATERIALS AND METHODS:* Longitudinal serum samples were taken while the patient was on study drugs. Serum concentration of VEGF, sVEGFR-3, and Ang-2 was determined by ELISA. The slope of the markers was correlated to the PFS. *RESULTS:* Patients showing a decrease in VEGF concentration had a median PFS of 10.5 months [confidence interval (CI), 2.89–12.25] compared to 2.9 months (CI, 1.48–5.32) in the case of an increase ($P = .17$). The stratified log-rank test showed a trend for longer PFS if a decrease of Ang-2 was observed ($P = .089$). Dichotomized in absolute decrease or increase, the PFS was 8.4 months (CI, 2.89–12.26) *versus* 2.7 months (CI, 1.05–5.32), respectively. Patients with a reduction of the sVEGFR-3 concentration had a median PFS of 4.76 months (CI 2.86–10.65) *versus* 8.61 months (CI, 1.05–not estimable) in patients with an increase of sVEGFR-3. This observation was statistically not significant in the log-rank test ($P = .81$). *CONCLUSION:* Ang-2 could potentially identify a patient population that might have a better PFS when under anti-angiogenic treatment, like the tyrosine kinase inhibitor sunitinib.

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Introduction

Ovarian cancer is the second most deadly gynecological malignancy with more than 22,000 new cases each year and more than 16,000 deaths per year [1] in the United States. In Germany, about 8000 new cases and more than 5500 deaths are reported each year [2]. Most patients are diagnosed at an advanced International Federation of Gynecology and Obstetrics (FIGO) stage III/IV and only 20% of those patients have a long-term survival. Cytoreductive surgery followed by combination chemotherapy of carboplatin and paclitaxel is considered as standard of care [3,4]. Despite initial response rates of up to 75%, the majority of patients experience a recurrence. Approximately 25% of patients develop platinum-resistant recurrence, defined as no response to platinum-based treatment or, after initial response, a recurrence within 6 months after the last platinum treatment. In this situation, therapeutic options are limited. To improve patients' symptoms and quality of life and potentially progression-free survival (PFS) and overall survival, new drugs or combinations are needed.

There is increasing interest in developing angiogenesis-suppressive agents for ovarian cancer treatment and a growing number of antiangiogenesis drugs are currently being evaluated in clinical trials for ovarian cancer. Bevacizumab, the monoclonal antibody targeting the vascular endothelial growth factor (VEGF), has shown to increase the PFS in recurrent platinum-sensitive ovarian cancer cases significantly [5]. In addition, the incorporation of bevacizumab in the first-line treatment of advanced ovarian cancer showed positive effects prolonging PFS [6,7] and maybe even overall survival in high-risk patients. Moreover, in women with platinum-resistant ovarian cancer, targeting VEGF by bevacizumab combined with standard-of-care chemotherapy improves the PFS compared to chemotherapy alone [8].

The AGO-OVAR2.11 study is a randomized phase II trial to evaluate the objective response rate to sunitinib in recurrent platinum-resistant ovarian cancer (EudraCT No. 2007-003089-16; ClinicalTrials.gov Identifier: NCT 00543049). A selection design was applied to compare two schedules of sunitinib: arm 1—50 mg sunitinib daily orally for 28 days followed by 14 days off drug and arm 2—37.5 mg sunitinib daily continuously. The results have been published recently [9]. The conclusion was that sunitinib shows activity in platinum-resistant ovarian cancer and that the noncontinuous schedule seems to be more effective.

The orally available multityrosine kinase inhibitor sunitinib (SU11248) shows anti-angiogenic activity and is approved for treating advanced stage or recurrent renal cell carcinoma [10] as well as imatinib-resistant gastrointestinal stromal tumors [11]. In a platinum-resistant ovarian cancer *in vivo* model, sunitinib shows a significant reduction in tumor progression [12]. Sunitinib interacts among other tyrosine kinases with the platelet derived growth factor (PDGF) receptor and the VEGFR, which are both expressed in ovarian cancer [13–16].

One major problem is to identify the right drug for the appropriate patients. Another unanswered question is how to monitor the biologic effect of these new drugs. The classic way to assess efficacy is to monitor tumor regression by radiographic imaging. However, especially for new types of drugs, such as anti-angiogenic drugs, it seems to be important to establish and validate molecular, cellular, or functional surrogate markers to monitor the activity and efficacy of anti-angiogenic compounds [17].

Angiopoietin-2 (Ang-2) is upregulated by tumor-derived VEGF in ovarian cancer, destabilizes the host vascular structure [18], and is already described in ovarian cancer. Soluble VEGF receptor-3 (sVEGFR-3) overexpression was found in high-risk ovarian cancer patients predicting poor outcome [19]. Patients with metastatic colorectal cancer showed a reduction in the sVEGFR-3 concentrations under sunitinib therapy [20]. In this translational project of the AGO-OVAR2.11 study, we determined the differential levels of VEGF, sVEGFR-3, and Ang-2 in sera of study participants longitudinally. VEGF has been studied quite extensively in patients treated with bevacizumab in various types of cancer [21,22] but failed to be of predictive value for response to this specific drug. Serum levels of VEGF, sVEGFR-3, and Ang-2 in recurrent ovarian cancer patients before and during the course of the targeted therapy were determined.

Materials and Methods

The AGO-OVAR2.11 trial was a multicenter, two-schedule and dose level, randomized (1:1), open label phase II study in patients with ovarian cancer resistant or refractory to platinum-based chemotherapy. Women who were 18 years or older and suitable for study participation according to the inclusion and exclusion criteria were enrolled after giving their signed and dated informed consent. The study protocol and informed consent form were reviewed and approved by an Independent Ethics Committee at each study center. The study was conducted by the Arbeitsgemeinschaft Gynäkologische Onkologie (AGO) Study Group in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The study was designed in accordance with the European Medicines Agency recommendations for clinical studies in cancer patients [23] (EudraCT No. 2007-003089-16. ClinicalTrials.gov Identifier: NCT 00543049).

Seventy-three patients were randomized (1:1 ratio) to receive either orally 50 mg sunitinib per os (p.o.) once daily for 4 weeks of every 6-week cycle [= noncontinuous group = arm 1 (n = 36)] or 37.5 mg sunitinib p.o. once daily continuously on a 6-week cycle [= continuous group = arm 2 (n = 37)]. The evaluation of response was based on the RECIST 1.0 criteria [24] in patients with measurable lesions. Fifty-eight of 73 (79.5%) patients had a measurable disease. In patients without measurable lesions, response rate was evaluated on the basis of the tumor marker CA125 serum concentration profiles according to the recommendations by the Gynecologic Cancer InterGroup (GCIG) [25,26]; serum collection was performed on each study visit. The objective response rate and the GCIG response shared the pattern. For the translational project, each patient signed an independent inform consent.

From 43 patients, serum samples were collected, and in 29 cases, at least two serum samples were obtained in intervals as stated in the study protocol; first interval was 28 days, thereafter 21 days. Baseline characteristics are summarized in Table 1.

Table 1. Baseline Characteristics of Patients.

	Entire Study Population (n = 73)	TR Population (n = 29)
Mean age (years)	58.6	55.4
PS ECOG 0/1 (n, %)	72 (98.6)	29 (100)
FIGO (n, %)		
I–IIA	4 (5.5)	0
IIB–III	55 (75.3)	20 (69)
IV	14 (19.2)	9 (31)
Histology (n, %)		
Serous	57 (78.1)	24 (82.8)
Endometrioid	5 (6.9)	2 (6.9)
Mucinous	3 (4.1)	0
Other	8 (10.9)	3 (10.3)
Postoperative tumor burden (n, %)		
0 cm	25 (34.3)	12 (41.4)
<1 cm	25 (34.3)	12 (41.4)
>1 cm	17 (23.3)	5 (17.2)

PS ECOG indicates Performance Status by the Eastern Cooperative Oncology Group.

Serum samples were collected prospectively; the scientists were blinded to the outcome of the individual patient. Serum samples were stored at −20°C until ELISA procedures were conducted in duplicates.

Protein concentrations of VEGF (Quantikine; Human VEGF Immunoassay DVE00; R&D Systems Europe, Wiesbaden-Nordenstadt, Germany), sVEGFR-3 (Human VEGFR3 DY349; R&D Systems Europe), and Ang-2 (Quantikine; Human Angiopoioetin-2 Immunoassay DANG20; R&D Systems Europe) were measured using ELISA according to the manufacturer's protocol. In brief, for each ELISA, a standard curve was generated and each sample was measured in duplicates after 10-fold dilution. One hundred microliters of assay diluents and 100 µl of diluted serum were added to each ELISA well and incubated for 2 hours. After washing, 200 µl of VEGF/sVERGr-3/Ang-2 conjugate was added to the well, respectively, followed by a 2-hour incubation. After repeated washing steps, 200 µl of substrate solution was added and incubated for 25 minutes. Fifty microliters of stop solution was given into each well. Optical density was determined within 30 minutes using a microplate reader set to 450 nm with wavelength correction at 540 nm.

Statistical analyses were done using SAS version 9.2.

Results

From the 73 participants, 43 individual patients gave their informed consent to collect serum samples. At least two samples were available for 29 patients, with a median age of 55.4 years (27–70 years). All patients had histologic grade 2 or 3 tumors and were FIGO stage IIIB to IV at initial diagnosis. With respect to the postoperative tumor burden at initial surgery, 44.4% had no macroscopic tumor and 55.6% had postoperative tumor residuals. Thirteen (44.8%) patients were randomized to the noncontinuous group, and 16 (55.2%) were treated continuously with sunitinib.

The first value of the investigated serum markers VEGF, sVEGFR-3, and Ang-2 served as a baseline parameter when the serum concentration was correlated to the patients' outcome. Using the baseline marker separately as a continuous covariate (Cox regression), no prognostic prediction in terms of PFS was found (Table 2).

For the most targeted therapies, no biomarker predicting the likely response to the drug is available. To evaluate the predictive potential of VEGF, sVEGFR-3, and Ang-2 as biomarkers during the course of therapy, we looked at the continuous differences between the baseline value and last available value of each patient in a Cox regression model. Again, no significant results were observed (Table 3).

There were also no significant differences ($P > .05$) found after splitting the cohorts according to the treatment arm.

Another aspect was if an absolute increase or decrease between the first and last available values might be helpful in identifying potential prognostic makers for PFS. For that purpose, the population of patients joining the biomarker program was divided into two groups: In the case of VEGF, an increase was observed in 11 cases (37.9%), and 18 patients (62.1%) showed a reduced serum concentration of VEGF.

Patients showing a decrease in VEGF concentration receiving sunitinib had a median PFS of 10.5 months [confidence interval (CI), 2.89–12.25] compared to 2.9 months (CI, 1.48–5.32) in case of an increase. In this post hoc analysis, the univariate log-rank test showed no significance [$P = .17$; hazard ratio (HR), 4.37; CI, 0.44–43.1].

Ang-2 might serve as a surrogate marker to predict PFS in recurrent platinum-resistant ovarian cancer, since sunitinib itself is not targeting this specific protein but has influences on the neoangiogenic potency that in part is reflected by Ang-2 levels.

In the investigated cohort, 21 patients showed a lower level during the course of therapy and 8 had higher levels of Ang-2 measured in the serum.

Dichotomized in absolute decrease or increase, the PFS was 8.4 months (CI, 2.89–12.26) *versus* 2.7 months (CI, 1.05–5.32), respectively. The stratified log-rank test showed a trend for longer PFS if a decrease of Ang-2 was observed ($P = .089$; HR, 1.29; CI, 0.01–not estimable).

The sVEGFR-3 was recently described as a potential predictive marker; therefore, we measured the serum levels in the study population. sVEGFR-3 was found to be decreased in 86% of patients ($n = 25$) and only 14% ($n = 4$) showed an increase. Patients with a reduction of the sVEGFR-3 concentration had a median PFS of 4.76 months (CI, 2.86–10.65) *versus* 8.61 months (CI, 1.05–not estimable) in patients with an increase of sVEGFR-3. This observation was statistically not significant in the log-rank test ($P > .8$; HR, 0.71; CI, 0.04–11.8; Table 4).

Since two dosing schedules—noncontinuous and continuous dosing—were used in this clinical trial, we analyzed the subgroup of patients taking part in translational project with respect to the potential influence of this actuality on the biomarker performance. No significant differences using the univariate chi-squared test were observed ($P = .68$).

Discussion

Tumor cells are capable of producing angiogenic factors to promote neoangiogenesis and to avoid tumor hypoxia. Angiogenesis is driven mainly by VEGF secreted among others by tumor cells and

Table 2. Results of the Cox Regression Model Evaluating the Baseline Values of Biomarkers ($n = 29$).

	Standard Error	Pr > ChiSq	Hazard Ratio	95% Confidence Limits	
VEGF	<0.001	0.2112	1.001	1.000	1.002
Ang-2	<0.001	0.8130	1.000	1.000	1.001
sVEGFR-3	<0.001	0.3889	1.000	1.000	1.000

Table 3. Results of the Cox Regression Model Evaluating the Difference between Baseline and Last Available Value of Biomarkers ($n = 29$).

	Standard Error	Pr > ChiSq	Hazard Ratio	95% Confidence Limits	
Diff_VEGF	0.003	0.4357	0.998	0.991	1.004
Diff_Ang-2	<0.001	0.1094	1.001	1.000	1.003
Diff_sVEGFR-3	<0.001	0.2960	1.000	0.999	1.000

Table 4. Median PFS in Patients with an Increase or Decrease in VEGF, Ang-2, or sVEGFR-3 ($n = 29$).

	Median PFS (Months)		*P* Value	HR
	Increase (*n*)	Decrease (*n*)		
VEGF	2.9 (11)	10.5 (18)	.17	4.37
Ang-2	2.7 (8)	8.4 (21)	.089	1.29
sVEGFR-3	4.8 (4)	8.6 (25)	.8	0.71

VEGFR-positive endothelial cells. Once the tumor reaches a critical size, it does need direct blood supply; therefore, the preexisting vascular system needs to be remodeled. VEGF plays a major role in ovarian cancer progression and metastases [27]. Targeting VEGF in preclinical [28] as well as in clinical settings [29] with platinum refractory ovarian cancer seems to be a promising option. Additionally, high expression of VEGF correlates with poor disease-free and overall survival in early-stage ovarian cancer [30]. Enhanced angiogenesis is indicated by high levels of VEGF and high microvessel density and is correlated with the presence of metastases and survival [31]. Yamamoto et al. [32] found that VEGF contributes to tumor progression in the vast majority of ovarian tumors. All patients in this investigation showed expression of VEGF at baseline, but the VEGF level was not predictive in terms of PFS. These findings are in line with previous results in patients with recurrent ovarian cancer treated with bevacizumab [21]. The authors also looked at VEGF in corresponding plasma and did not find a difference in PFS and overall survival for baseline values. When looking at the course of the VEGF levels under therapy, it emerged in this study that patients with decreasing levels had a better PFS compared to those with an increase, 10.5 months *versus* 2.9 months, respectively. These findings are contradictory to the findings by Han et al., which might be in part explained by the different drugs used. However, the results from serum VEGF should be interpreted with caution since at least for bevacizumab treatment a potential interference is reported [33]. In a comprehensive analysis, Hedge et al. [22] found that the baseline plasma VEGF-A level is of prognostic value in metastatic colorectal, lung, and renal cancers with patients with high levels showing shorter overall survival. However, this investigation could not show a predictive value of plasma VEGF-A levels for bevacizumab-based treatment benefit.

Next to VEGF, angiopoietins (Ang-1 and Ang-2) are heavily involved in tumor neovascularization. Angiopoietins are secreted and can be measured by ELSIA tests in body fluids, e.g., patients' serum and/or ascites [34]. Ang-1 guards the vascular stability and architecture by promoting the adhesive interactions between endothelial cells and surrounding cells. Ang-2—being an Ang-1 antagonist—in turn destabilizes the host vasculature in the presence of VEGF and supports angiogenesis [35]. Ang-2 solely does promote vascular regression [36,37]. In gastric cancer patients, high Ang-2 mRNA levels reflects advanced tumor stage and poorer prognosis [38]. A high Ang-2/Ang-1 mRNA ratio is associated with poorer outcome in patients with hepatocellular carcinomas [39].

Zhang et al. [18] described the Ang-2 up-regulation and host vascular destabilization by tumor-derived VEGF in ovarian cancer. They found that Ang-2 was almost exclusively expressed by endothelial cells of blood vessels localized in the tumor stroma; only a minority of cancer cells expressed Ang-2 themselves. Tumor cells overexpressing VEGF are triggering tumor vasculogenesis by Ang-2. In *in vitro* experiments, VEGF induces Ang-2 transcription through VEGFR-2 (KDR) and can be blocked be a specific KDR inhibitor [18]. This cross talk emphasizes Ang-2 as a very interesting molecule to investigate in anti-angiogenic therapy, especially in conjunction with sunitinib since this molecule interferes with the receptor KDR/VEGFR-2 that promotes Ang-2 transcription. Ang-2 seems to be more meaningful in screening actual vasculogenesis because it is transcripted and translated in endothelial cells. By immunohistochemistry and immunofluorescence, Zhang et al. localized Ang-2 expression to CD31-positive endothelial cells and only to a few ovarian cancer cells itself. Measuring VEGF levels would only provide indirect inference because it gets mostly expressed by the tumor cell itself. A hypothesis could even be that the VEGF level would increase short term in consequence of reduced blood supply. Destabilization in tumors with VEGF and Ang-2 overexpression lead to disorganized large and medium caliber blood vessels with significant pericyte loss. No changes could be detected in capillaries since those are without a pericyte layer [18]. In their investigation, the Ang-2 value at baseline was not predictive for the patients' outcome. However, in our analysis, decreased Ang-2 levels were accompanied by a trend for longer PFS (8.4 *vs* 2.7 months, $P = .0896$).

Clinical studies using sunitinib in patients with different types of metastatic cancer revealed members of the soluble component of the VEGFR family as a potential surrogate marker (reviewed in [40]). In metastatic renal cancer, a decline of sVEGFR-3 concentration was correlated to a better outcome [41]. In contrast—although not statistically significant ($P > .8$)—we show that patients with an increase in sVEGFR-3 levels had a better PFS under sunitinib therapy than those with declining serum levels, 8.6 months *versus* 4.8 months, respectively. In an ovarian cancer mouse model, adenovirus-mediated gene transfer with sVEGFR-1, sVEGFR-2, and sVEGFR-3 in combination with paclitaxel was more effective than, e.g., the combination of paclitaxel and bevacizumab [42]. In general, it is important to learn more about the function and the impact of sVEGFR-3.

With respect to clinicopathologic criteria such as histologic subtype and residual tumor after initial surgery, no statistical analyses were done since the sample size was already small.

In the AGO-OVAR2.11 study, the residual tumor after initial surgery was not considered for statistical evaluation. Most patients (89%) within the study had received more than one chemotherapy treatment before the study drug. Whether the residual tumor burden is of any impact for second-line and third-line therapies remains unknown; furthermore, the study had no statistical power to address this issue. Whether the histologic subtype would have had any impact on the response rate is still unclear. In the AGO-OVAR2.11 study, the most common histologic subtype was the serous subtype, total of 57 patients (78%). All other subtypes were less frequent; therefore, a subgroup analysis was neither performed in the AGO-OVAR2.11 study cohort nor in the translational project cohort. Whether the residual tumor burden or the histologic subtype is of any impact for second-line and third-line therapies remains unknown; furthermore, the AGO-OVAR2.11 study was conducted as a phase II trial and therefore had no statistical power to address this issue.

The presented study has limitations with respect to sample size and the exploratory nature of the investigation. The number of patients within the study and the number of samples of the translational project are small due to multiple reasons such as advanced tumor affection and therapy-related toxicity. This highlights the difficulties in conducting biomarker correlative studies. The translational project was started to identify potential surrogate markers to confirm them in larger study populations. It seems to be most promising to follow-up on Ang-2, although the differences were not significant. This marker could potentially identify a patient population that might have a better PFS when under anti-angiogenic treatment, like the tyrosine kinase inhibitor sunitinib. In general, it might be more successful using multiple markers than single ones. However, it is the challenge for the future to identify at least one specific marker or even a marker panel that could predict response to a targeted therapy such as sunitinib. However, to test for marker combinations, the sample size in clinical trials has to be high enough to provide adequate statistical power.

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References

[1] Siegel R, Naishadham D, and Jemal A (2012). Cancer statistics, 2012. *CA Cancer J Clin* **62**, 10–29.

[2] Robert Koch-Institut and Deutschland GdeKi (2012). *Krebs in Deutschland 2007/2008* (8th ed). Robert Koch-Institut und die Gesellschaft der epidemiologischen Krebsregister in Deutschland e.V.

[3] Bristow RE, Tomacruz RS, Armstrong DK, Trimble EL, and Montz FJ (2002). Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis. *J Clin Oncol* **20**, 1248–1259.

[4] du Bois A, Luck HJ, Meier W, Adams HP, Mobus V, Costa S, Bauknecht T, Richter B, Warm M, Schroder W, et al. (2003). A randomized clinical trial of cisplatin/paclitaxel versus carboplatin/paclitaxel as first-line treatment of ovarian cancer. *J Natl Cancer Inst* **95**, 1320–1329.

[5] Aghajanian C, Blank SV, Goff BA, Judson PL, Teneriello MG, Husain A, Sovak MA, Yi J, and Nycum LR (2012). OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. *J Clin Oncol* **30**, 2039–2045.

[6] Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, Mannel RS, Homesley HD, Fowler J, Greer BE, et al. (2011). Incorporation of bevacizumab in the primary treatment of ovarian cancer. *N Engl J Med* **365**, 2473–2483.

[7] Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G, Carey MS, Beale P, Cervantes A, Kurzeder C, et al. (2011). A phase 3 trial of bevacizumab in ovarian cancer. *N Engl J Med* **365**, 2484–2496.

[8] Pujade-Lauraine E, Hilpert F, Weber B, Reuss A, Poveda A, Kristensen G, Sorio R, Vergote IB, Witteveen P, Bamias A, et al. (2012). AURELIA: a randomized phase III trial evaluating bevacizumab (BEV) plus chemotherapy (CT) for platinum (PT)-resistant recurrent ovarian cancer (OC). *J Clin Oncol* **30**(suppl abstr LBA5002).

[9] Baumann KH, du Bois A, Meier W, Rau J, Wimberger P, Sehouli J, Kurzeder C, Hilpert F, Hasenburg A, Canzler U, et al. (2012). A phase II trial (AGO 2.11) in platinum-resistant ovarian cancer: a randomized multicenter trial with sunitinib (SU11248) to evaluate dosage, schedule, tolerability, toxicity and effectiveness of a multitargeted receptor tyrosine kinase inhibitor monotherapy. *Ann Oncol* **23**, 2265–2271.

[10] Motzer RJ, Rini BI, Bukowski RM, Curti BD, George DJ, Hudes GR, Redman BG, Margolin KA, Merchan JR, Wilding G, et al. (2006). Sunitinib in patients with metastatic renal cell carcinoma. *JAMA* **295**, 2516–2524.

[11] Goodman VL, Rock EP, Dagher R, Ramchandani RP, Abraham S, Gobburu JV, Booth BP, Verbois SL, Morse DE, Liang CY, et al. (2007). Approval summary: sunitinib for the treatment of imatinib refractory or intolerant gastrointestinal stromal tumors and advanced renal cell carcinoma. *Clin Cancer Res* **13**, 1367–1373.

[12] Bauerschlag DO, Schem C, Tiwari S, Egberts JH, Weigel MT, Kalthoff H, Jonat W, Maass N, and Meinhold-Heerlein I (2010). Sunitinib (SU11248) inhibits growth of human ovarian cancer in xenografted mice. *Anticancer Res* **30**, 3355–3360.

[13] Arora A and Scholar EM (2005). Role of tyrosine kinase inhibitors in cancer therapy. *J Pharmacol Exp Ther* **315**, 971–979.

[14] Wilczynski SP, Chen YY, Chen W, Howell SB, Shively JE, and Alberts DS (2005). Expression and mutational analysis of tyrosine kinase receptors c-kit, PDGFRα, and PDGFRβ in ovarian cancers. *Hum Pathol* **36**, 242–249.

[15] Lassus H, Sihto H, Leminen A, Nordling S, Joensuu H, Nupponen NN, and Butzow R (2004). Genetic alterations and protein expression of KIT and PDGFRA in serous ovarian carcinoma. *Br J Cancer* **91**, 2048–2055.

[16] Boocock CA, Charnock-Jones DS, Sharkey AM, McLaren J, Barker PJ, Wright KA, Twentyman PR, and Smith SK (1995). Expression of vascular endothelial growth factor and its receptors flt and KDR in ovarian carcinoma. *J Natl Cancer Inst* **87**, 506–516.

[17] Ledermann JA, Marth C, Carey MS, Birrer M, Bowtell DD, Kaye S, McNeish I, Oza A, Scambia G, Rustin G, et al. (2011). Role of molecular agents and targeted therapy in clinical trials for women with ovarian cancer. *Int J Gynecol Cancer* **21**, 763–770.

[18] Zhang L, Yang N, Park JW, Katsaros D, Fracchioli S, Cao G, O'Brien-Jenkins A, Randall TC, Rubin SC, and Coukos G (2003). Tumor-derived vascular endothelial growth factor up-regulates angiopoietin-2 in host endothelium and destabilizes host vasculature, supporting angiogenesis in ovarian cancer. *Cancer Res* **63**, 3403–3412.

[19] Klasa-Mazurkiewicz D, Jarzab M, Milczek T, Lipinska B, and Emerich J (2011). Clinical significance of VEGFR-2 and VEGFR-3 expression in ovarian cancer patients. *Pol J Pathol* **62**, 31–40.

[20] Kanefendt F, Lindauer A, Mross K, Fuhr U, and Jaehde U (2012). Determination of soluble vascular endothelial growth factor receptor 3 (sVEGFR-3) in plasma as pharmacodynamic biomarker. *J Pharm Biomed Anal* **70**, 485–491.

[21] Han ES, Burger RA, Darcy KM, Sill MW, Randall LM, Chase D, Parmakhtiar B, Monk BJ, Greer BE, Connelly P, et al. (2010). Predictive and prognostic angiogenic markers in a gynecologic oncology group phase II trial of bevacizumab in recurrent and persistent ovarian or peritoneal cancer. *Gynecol Oncol* **119**, 484–490.

[22] Hegde PS, Jubb AM, Chen D, Li NF, Meng YG, Bernaards C, Elliott R, Scherer SJ, and Chen DS (2013). Predictive impact of circulating vascular endothelial growth factor in four phase III trials evaluating bevacizumab. *Clin Cancer Res* **19**, 929–937.

[23] (CPMP) CfPMP (2002). *Note for Guidance on Evaluation of Anticancer Medicinal Products In Man*. EMEA, London. CPMP/EWP/205/95rev.2-corr.

[24] Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, et al. (2000). New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* **92**, 205–216.

[25] Gynecologic Cancer InterGroup (2006). CA 125 Definitions Agreed by GCIG November 2005. Available at: http://ctep.cancer.gov/resources/gcig/srespdef_nov2005.doc.

[26] Rustin GJ, Timmers P, Nelstrop A, Shreeves G, Bentzen SM, Baron B, Piccart MJ, Bertelsen K, Stuart G, Cassidy J, et al. (2006). Comparison of CA-125 and standard definitions of progression of ovarian cancer in the intergroup trial of cisplatin and paclitaxel versus cisplatin and cyclophosphamide. *J Clin Oncol* **24**, 45–51.

[27] Li L, Wang L, Zhang W, Tang B, Zhang J, Song H, Yao D, Tang Y, Chen X, Yang Z, et al. (2004). Correlation of serum VEGF levels with clinical stage, therapy efficacy, tumor metastasis and patient survival in ovarian cancer. *Anticancer Res* **24**, 1973–1979.

[28] Rein DT, Volkmer AK, Volkmer J, Beyer IM, Janni W, Fleisch MC, Welter AK, Bauerschlag D, Schondorf T, and Breidenbach M (2012). Systemic administration of bevacizumab prolongs survival in an *in vivo* model of platinum pre-treated ovarian cancer. *Oncol Lett* **3**, 530–534.

[29] Verschraegen CF, Czok S, Muller CY, Boyd L, Lee SJ, Rutledge T, Blank S, Pothuri B, Eberhardt S, and Muggia F (2012). Phase II study of bevacizumab with liposomal doxorubicin for patients with platinum- and taxane-resistant ovarian cancer. *Ann Oncol* **23**, 3104–3110.

[30] Paley PJ, Staskus KA, Gebhard K, Mohanraj D, Twiggs LB, Carson LF, and Ramakrishnan S (1997). Vascular endothelial growth factor expression in early stage ovarian carcinoma. *Cancer* **80**, 98–106.

[31] Bamberger ES and Perrett CW (2002). Angiogenesis in epithelian ovarian cancer. *Mol Pathol* **55**, 348–359.

[32] Yamamoto S, Konishi I, Mandai M, Kuroda H, Komatsu T, Nanbu K, Sakahara H, and Mori T (1997). Expression of vascular endothelial growth factor (VEGF) in epithelial ovarian neoplasms: correlation with clinicopathology and patient survival, and analysis of serum VEGF levels. *Br J Cancer* **76**, 1221–1227.

[33] Loupakis F, Falcone A, Masi G, Fioravanti A, Kerbel RS, Del Tacca M, and Bocci G (2007). Vascular endothelial growth factor levels in immunodepleted plasma of cancer patients as a possible pharmacodynamic marker for bevacizumab activity. *J Clin Oncol* **25**, 1816–1818.

[34] Sallinen H, Heikura T, Laidinen S, Kosma VM, Heinonen S, Yla-Herttuala S, and Anttila M (2010). Preoperative angiopoietin-2 serum levels: a marker of malignant potential in ovarian neoplasms and poor prognosis in epithelial ovarian cancer. *Int J Gynecol Cancer* **20**, 1498–1505.

[35] Benjamin LE, Golijanin D, Itin A, Pode D, and Keshet E (1999). Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. *J Clin Invest* **103**, 159–165.

[36] Holash J, Maisonpierre PC, Compton D, Boland P, Alexander CR, Zagzag D, Yancopoulos GD, and Wiegand SJ (1999). Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. *Science* **284**, 1994–1998.

[37] Lobov IB, Brooks PC, and Lang RA (2002). Angiopoietin-2 displays VEGF-dependent modulation of capillary structure and endothelial cell survival *in vivo*. *Proc Natl Acad Sci USA* **99**, 11205–11210.

[38] Etoh T, Inoue H, Tanaka S, Barnard GF, Kitano S, and Mori M (2001). Angiopoietin-2 is related to tumor angiogenesis in gastric carcinoma: possible *in vivo* regulation via induction of proteases. *Cancer Res* **61**, 2145–2153.

[39] Mitsuhashi N, Shimizu H, Ohtsuka M, Wakabayashi Y, Ito H, Kimura F, Yoshidome H, Kato A, Nukui Y, and Miyazaki M (2003). Angiopoietins and Tie-2 expression in angiogenesis and proliferation of human hepatocellular carcinoma. *Hepatology* **37**, 1105–1113.

[40] DePrimo SE and Bello C (2007). Surrogate biomarkers in evaluating response to anti-angiogenic agents: focus on sunitinib. *Ann Oncol* **18**(suppl 10), x11–x19.

[41] Deprimo SE, Bello CL, Smeraglia J, Baum CM, Spinella D, Rini BI, Michaelson MD, and Motzer RJ (2007). Circulating protein biomarkers of pharmacodynamic activity of sunitinib in patients with metastatic renal cell carcinoma: modulation of VEGF and VEGF-related proteins. *J Transl Med* **5**, 32.

[42] Sopo M, Anttila M, Sallinen H, Tuppurainen L, Laurema A, Laidinen S, Hamalainen K, Tuunanen P, Koponen JK, Kosma VM, et al. (2012). Anti-angiogenic gene therapy with soluble VEGF-receptors -1, -2 and -3 together with paclitaxel prolongs survival of mice with human ovarian carcinoma. *Int J Cancer* **131**, 2394–2401.