Prognostic Significance of Angiogenic Factors in Uterine Cervical Cancer

SOLVEIG LANDT[1], MICHAEL WEHLING[2], HARALD HEIDECKE[3], SOPHIA JESCHKE[4], SUSANNE KORLACH[†], FRANK STÖBLEN[5], PETER SCHMID[6], JENS-UWE BLOHMER[7], WERNER LICHTENEGGER[4], JALID SEHOULI[4] and SHERKO KÜMMEL[2]

[1]*Department of Gynecology and Obstetrics, University Hospital Düsseldorf, Düsseldorf, Germany;*
[2]*Breast Center, and* [5]*Department of Radiology, Huyssensstift Kliniken Essen-Mitte, Essen, Germany;*
[3]*CellTrend GmbH, Luckenwalde, Germany;*
[4]*Department of Gynecology and Obstetrics, Charité, University Hospital Berlin, Berlin, Germany;*
[6]*Brighton and Sussex Medical School, Trafford Centre, University of Sussex, Brighton, UK;*
[7]*Department of Gynecology and Obstetrics, Sankt Gertrauden Hospital, Berlin, Germany*

Abstract. *Background/Aim: Angiogenesis is pivotal in tumour development and progress, and targeted tumour therapies, such as bevacizumab, have shown promising results. However, in unselected patient populations, the treatment with angiogenesis-targeted combination regimens is marred by a variable response, non-negligible toxicity and questionable economy. The present study summarizes research to identify individual circulating angiogenic factors as markers for disease severity and possibly treatment response. Patients and Methods: A total of 125 patients with cervical cancer from the ongoing cervical cancer monitoring database of the University Hospital Charité, Berlin, Germany, were included. Information obtained from the database included tumour stage, malignancy grade, presence of nodal metastases, lymph vessel invasion, patient age, HER2, HPV, smoking and menopausal status, and serum concentrations of vascular endothelial growth factor (VEGF), VEGF-D, VEGF-C, endoglin, endostatin, angiogenin, basic fibroblast growth factor (FGFb), vascular endothelial growth factor receptor (VEGF-R1), VEGF-R2, soluble inter-cellular adhesion molecule 1 (sICAM 1), soluble vascular adhesion molecule 1 (sVCAM 1), insulin-like growth factor 1 (IFG-1) and insulin like growth factor binding protein 3 (IGF-BP3). Results: There was a clear association of angiogenic factor concentrations with stage of disease. Angiogenin showed an independent discrimination for cervical intraepithelial neoplasia (CIN) and invasive stages, and endoglin did so for invasive stages vs. recurrent disease. However, none of the potential markers under investigation was anywhere near selective enough to allow for a clinically meaningful prediction of prognosis or response. Conclusion: The association of circulating angiogenic factors with disease progression in cervical cancer is confirmed, but its utility for prognosis prediction and patient stratification for targeted therapies is doubtful.*

Despite substantial advances due to earlier diagnosis and improved treatment methods, uterine cervical cancer remains a globally leading cause of cancer death in females; there are in excess of 500,000 new cases and more than 250,000 deaths per year (1, 2) (and probably many more due to under-reporting in many countries (3)). In the European Union, an estimated 60,000 new cases and 30,000 deaths occur per year (4), and in Germany, the annual number of fatal cases in recent years was around 1,500 (5, 6).

The clinical outcome of cervical cancer, albeit generally favourable provided comprehensive screening treatment is available (7), varies significantly and is difficult to predict for individual patients. Moreover, the availability of novel, angiogenesis-targeted treatment modalities has given rise to the search for diagnostic markers allowing a response prediction (8, 15), both in the interest of cost efficiency and an improved effect toxicity balance. Diagnostic methods that allow an accurate prognosis and response assessment would be immensely valuable for the implementation of individualised treatment modalities.

Angiogenesis is a validated target for specific therapeutic modalities currently under investigation, and

[†]Retired.

Correspondence to: Solveig Landt, Department of Gynecology and Obstetrics, University Hospital Düsseldorf, Moorenstrasse 5, 40225 Düsseldorf, Germany. Tel: +49 1622387535, e-mail: solveiglandt@yahoo.de

Key Words: Angiogenic factors, cervical cancer, VEGF, angiogenin, endoglin.

agents against established and novel targets for a variety of malignancies, including cervical cancer, are under intense development (9, 14, 16). One of the most promising targets for such treatments is angiogenesis, which is crucially involved in tumour growth, invasion, and metastasis (14, 16, 17) and has been shown to be induced by cervical neoplasms (18, 19). The currently most intensively investigated angiogenic-targeted agent is the monoclonal antibody bevacizumab, which has evolved as an established modality for several types of cancer, including cervical cancer (8, 14, 20-23).

However, response to novel targeted treatment modalities is more variable than in conventional chemotherapy (24, 25), and aggressive combinatorial treatment approaches are associated with an increased risk of toxicity (26, 27). The systematic implementation of anti-angiogenic therapy requires a reliable identification of patients with a likely benefit (28-30), and validated treatment protocols with a proven efficacy and acceptable toxicity are not yet available. First of all, the currently available agents (such as bevacizumab (8, 14, 23) are too expensive to be incorporated into treatment regimens for unselected populations (31). Moreover, the long-term suppression of one angiogenic factor may induce the expression of one or more others by a linked alternative angiogenic pathway (so-called tolerance), which is potentially hazardous in patients in whom the suppressed factor was not a pivotal factor in the first place (9).

Therefore, a reliable identification of susceptible patients is a prerequisite for the implementation of angiogenesis-targeted treatment strategies. The present paper summarises a research programme conducted in 125 patients at the Charité, University Hospital Berlin, Germany. Its goal was to assess the statistical significance and independence of individual angiogenic factors that were associated with advanced stages of disease (12, 13).

Patients and Methods

Patients. The study participants represent a sample of patients from the ongoing cervical cancer monitoring database of the Charité, University Hospital Berlin, Germany. Data acquisition, storage and processing in this database required written informed consent hence no specific ethical requirements were considered for the present investigation. Patients who underwent diagnostic or follow-up examinations for cervical uterine neoplasms between October, 2002 and June, 2005 were enrolled into the study. A total of 125 patients were included, and their serum samples were obtained prior to therapy and stored at –80˚C immediately after collection.

Data acquisition. Information obtained from the database included tumour stage, histology, presence of nodal metastases, lymphatic and venous vessel invasion as well as patient age, menopausal and smoking status. The sample characteristics regarding the aforementioned criteria are shown in Table I.

Table I. *Baseline characteristics of patients included in this study.*

Criterion	Number	Percentage
Tumour stage		
Non-invasive	50	40.0
CIN I	7	5.6
CIN II	8	6.4
CIN III	35	28.0
Invasive	51	40.8
FIGO I	22	17.6
FIGO II	13	10.4
FIGO III	13	10.4
FIGO IV	3	2.4
Recurrent disease	24	19.2
Tumour histology		
(only invasive tumours)		
Squamous cell carcinoma	58	76.3
Adenocarcinoma	8	10.7
Adenosquamous carcinoma	5	6.7
Not classifiable	4	5.3
Prognostic criteria		
Nodal metastasis	29	23.2
Lymph vessel invasion	20	16.0
Blood vessel invasion	8	6.4
Grading		
G 1	2	1.6
G 2	38	30.4
G 3	31	24.8
Non available	54	43.2
Age, years (average)	42.9±13.5	
Menopausal status		
Pre-menopausal	88	70.4
Menopausal	2	1.6
Post-menopausal	35	28.0
Smoking status		
Smoker	45	36.0
Non-smoker	47	37.6
Not available	33	26.4

The serum concentrations of angiogenic factors (vascular endothelial growth factor (VEGF), -D, -C, -R1, -R2, angiogenin, endoglin and endostatin), adhesion molecules (soluble inter-cellular adhesion molecule 1 (sICAM 1), soluble vascular adhesion molecule 1 (s-VCAM 1)) and growth factors (insuline like growth factor 1 (IFG-1), insulin like growth factor binding protein 3 (IGF-BP3) were determined by ELISA (R&D Systems, Minneapolis, MN, USA) as part of the clinical routine, and the respective values were obtained from the database.

Statistical data evaluation. Data was stored in a Microsoft Excel™ spreadsheet and analysed with the SPSS™ 15.0 software package (SPSS™ Inc., Chicago, IL). Non-parametric methods were employed for analysis, and correlations between prognostic factors and tumour stage were assessed with one-factorial and multiple logistic regression; variables with a p-value ≤0.10 in one-factorial testing were included in the multiple regression. Odds ratios (adjusted odds ratios in multiple regression) and 95% confidence intervals (95% CI) were calculated, and independent statistical significance was considered when p was <0.05 in multiple regression.

Table II. *Assessed prognostic factors (mean±std. dev. or frequency and percentage) according to tumour stage.*

Variable	Entire sample (n=25)	CIN stages (n=50)	FIGO stages (n=50)	Relapse (n=24)
Age (years)	43.2±13.4	36.8±11.6	47.8±13.9	47.0±10.8
VEGF, plasma (pg/ml)	118.4±117.1	81.4±56.3	138.7±146.7	152.3±123.9
VEGF-D, serum (pg/ml)	398.7±261.9	425.8±170.3	382.5±332.8	376.7±254.2
VEGF-C, serum (ng/ml)	10.314±3.858	9.653±2.971	10.682±4.581	10.910±3.771
Endoglin, serum (ng/ml)	4.2±1.1	4.5±1.0	4.2±1.2	3.4±0.8
Endostatin, serum (ng/ml)	120.6±47.5	102.8±17.3	115.7±51.1	168.2±52.2
Angiogenin, serum (ng/ml)	326.5±101.6	276.7±69.6	336.1±101.7	410.1±100.0
FGFb, serum (pg/ml)	5.5±8.6	4.2±5.1	4.6±5.8	10.1±15.7
VEGF-R1, serum (pg/ml)	75.3±28.3	73.1±19.7	79.7±34.2	70.2±29.5
VEGF-R2, serum (ng/ml)	10.653±2.056	10.606±2.202	10.243±1.974	11.622±1.628
sICAM 1, serum (ng/ml)	325.0±120.9	297.0±77.7	317.6±104.8	401.0±187.6
sVCAM 1, serum (ng/ml)	661.3±335.6	585.5±111.7	716.1±486.6	701.3±193.1
IFG-1, serum (ng/ml)	127.8±48.4	133.8±44.9	119.3±51.8	134.2±47.1
IGF-BP3, serum (ng/ml)	2,776±702	2,816±482	2,586±843	3,114±640
Menopausal status				
Post-menopausal	35 (28.0%)	6 (12.0%)	19 (37.3%)	10 (41,7%)
Pre- or peri-menopausal	90 (72.0%)	44 (88.0%)	32 (62.7%)	14 (58.3%)
Smoking status†				
Smoker	45 (48.9%)	16 (32.0%)	22 (47.8%)	7 (41.2%)
Non-smoker	47 (51.1%)	13 (26.0%)	24 (52.2%)	10 (58.8%)
HPV status†				
High risk	28 (56.0%)	24 (64.9%)	4 (33.3%)	-
Low risk/negative	22 (44.0%)	13 (35.1%)	8 (66.7%)	1 (100.0%)
Distant metastasis†				
Yes	17 (14.0%)	0 (0.0%)	3 (6.1%)	14 (63.3%)
No	104 (86.0%)	50 (100.0%)	46 (93.9%)	8 (36.4%)

†Cases with missing values were excluded from analysis.

Results

The determined serum and plasma concentrations of the examined factors are shown in Table II. When comparing mean values between subgroups of CIN, FIGO and relapse, a plausible dependency on the stage (*i.e.* CIN<FIGO<relapse or *vice versa*) is apparent for the following variables: An increase for VEGF (with a particularly marked difference between CIN and FIGO stages), VEGF-C, endostatin, angiogenin and FGFb (with a particularly marked difference between FIGO stages and relapse), and sICAM-1; a decrease for VEGF-D and endoglin, the latter with a particularly marked difference between FIGO stages and relapse. Furthermore, the percentage of post-menopausal patients increased over stages, whereas those with high-risk HPV status decreased.

CIN vs. FIGO stages. When comparing pre-invasive (CIN I-III) and invasive (FIGO I-IV) tumour stages (Table III), the only significant prognostic criteria were age (about 10% increasing chance for invasive stages per year) and serum angiogenin concentration (1% per ng/ml). The aforementioned, relatively marked difference in plasma VEGF concentrations between CIN and FIGO stages led to a modest correlation (1% per pg/ml) that was, however, significant only in one-factorial but not in multiple testing.

FIGO stages vs. relapse. The aforementioned marked difference in endoglin proved to be an independent indicator for relapse: Per ng/ml endoglin decrease, the likelihood of a relapse increased by almost 20% (Table IV). The IGF-BP3 concentration showed a significant positive correlation with the chance of relapse, but had shown an inverse relation to CIN/FIGO stages; therefore, its informative value as a measure for tumour stage is limited.

Pre-invasive and invasive carcinoma subgroups. None of the examined factors (descriptive data not shown) allowed for an independent prediction of CIN III *vs*. CIN I/II or FIGO III/IV *vs*. FIGO I/II, respectively. The only parameter with a borderline significant discrimination between early and advanced invasive stages was that of VEGF-C (p=0.056 in multiple regression testing). Its concentration was substantially lower in advanced stage disease (7,998±1,831 [FIGO III/IV] and 11,909±4,943 [FIGO I/II] pg/ml serum); however, when compared with concentrations in pre-invasive stages (8,672±2,641 [CIN I/II] and 10,073±3,041 [CIN III] pg/ml

Table III. *Odds ratio (OR), adjusted odds ratio (aOR) and significance for individual factors, FIGO vs. CIN stages.*

Risk: FIGO stage	OR (95% CI)	*p*-value	aOR (95 %CI)	*p*-value
Age (years)*↑	1.08 (1.03-1.12)	0.0003	1.09 (1.02-1.16)	0.008
Plasma VEGF (pg/ml)	1.01 (1.00-1.01)	0.017	1.01 (1.00-1.01)	0.13
Serum VEGF-D (pg/ml)	1.00 (1.00-1.00)	0.43		
Serum VEGF-C (pg/ml)	1.00 (1.00-1.00)	0.19		
Serum Endoglin (ng/ml)	0.76 (0.52-1.11)	0.16		
Serum Endostatin (ng/ml)	1.01 (1.00-1.03)	0.12		
Serum Angiogenin (ng/ml)*↑	1.01 (1.00-1.01)	0.0021	1.01 (1.00-1.01)	0.012
Serum FGFb (pg/ml)	1.01 (0.94-1.09)	0.72		
Serum VEGF-R1 (pg/ml)	1.01 (0.99-1.02)	0.25		
Serum VEGF-R2 (pg/ml)	1.00 (1.00-1.00)	0.38		
Serum sICAM 1 (ng/ml)	1.00 (1.00-1.01)	0.28		
Serum sVCAM 1 (ng/ml)	1.00 (1.00-1.01)	0.067	1.00 (1.00-1.00)	0.22
Serum IFG-1 (ng/ml)	0.99 (0.98-1.00)	0.15		
Serum IGF-BP3 (ng/ml)	1.00 (1.00-1.00)	0.11		
Post-menopausal status	2.08 (1.25-3.45)	0.0049	0.37 (0.07–2.08)	0.26
Smoker	0.86 (0.54-1.37)	0.54		
High risk HPV	0.52 (0.26-1.03)	0.063		

*$p<0.05$ in multiple regression.

Table IV. *Odds ratio (OR), adjusted odds ratio (aOR) and significance for individual factors, FIGO stages vs. relapse.*

Risk: Relapse	OR (95 %CI)	*p*-value	aOR (95%CI)	*p*-value
Age (years)	1.00 (0.96-1.03)	0.80		
Plasma VEGF (pg/ml)	1.00 (1.00-1.00)	0.69		
Serum VEGF-D (pg/ml)	1.00 (1.00-1.00)	0.94		
Serum VEGF-C (pg/ml)	1.00 (1.00-1.00)	0.83		
Serum Endoglin (ng/ml)*↓	0.38 (0.20-0.71)	0.0023	0.17 (0.04-0.70)	0.014
Serum Endostatin (ng/ml)	1.02 (1.01-1.04)	0.0019	1.00 (0.98-1.02)	0.82
Serum Angiogenin (ng/ml)	1.01 (1.00-1.01)	0.0071	1.00 (0.99-1.01)	0.72
Serum FGFb (pg/ml)	1.07 (0.99-1.15)	0.078	1.05 (0.89-1.24)	0.56
Serum VEGF-R1 (pg/ml)	0.99 (0.97-1.01)	0.26		
Serum VEGF-R2 (pg/ml)	1.00 (1.00-1.00)	0.0072	1.00 (1.00-1.00)	0.26
Serum sICAM 1 (ng/ml)	1.00 (1.00-1.01)	0.046	1.01 (0.99-1.02)	0.25
Serum sVCAM 1 (ng/ml)	1.00 (1.00-1.00)	0.89		
Serum IFG-1 (ng/ml)	1.01 (1.00-1.02)	0.25		
Serum IGF-BP3 (ng/ml)*↑	1.00 (1.00-1.00)	0.016	1.00 (1.00-1.00)	0.048
Post-menopausal status	0.91 (0.56-1.50)	0.71		
Smoker	1.14 (0.65-2.01)	0.64		
High risk HPV	n/a (no high-risk HPV in group "relapse")			

*$p<0.05$ in multiple regression.

serum) and relapse (10,910±3773 pg/ml serum), the correlation with disease severity is hardly convincing.

Discussion

According to the Gynecologic Oncology Group, the development of novel treatment regimens is imperative, and anti-angiogenic modalities are promising (14, 15). Bevacizumab treatment is currently under investigation, albeit only in women eligible for second- or third-line treatment (15).

The present study basically corroborates the well-known fact that circulating concentrations of some angiogenic factors indeed reflect the degree of biological aggressiveness of uterine cervical cancer, and indicate a particular relevance of endoglin (see also 13, 32, 33) in addition to the more established VEGF (10, 12, 17, 19, 34, 35). However, it was not possible to single out an individual factor or a combination (and much less, a reasonably selective threshold) that would allow for a rational stratification of patients for novel targeted treatment modalities.

Circulating angiogenic factors have failed to reliably predict response to bevacizumab as yet. For instance, a recently published trial on bevacizumab treatment of colorectal cancer (36) showed no correlation of VEGF expression with treatment success. Most likely, bevacizumab acts by scavenging the VEGF released by conventional chemotherapy, and therefore a straightforward correlation would not necessarily be plausible. Moreover, antiangiogenic therapy can promote rather than diminish tumour progression and spread in some cases (37, 38), rendering a reliable identification of patients who will benefit from treatment crucial.

As a consequence of the hitherto futile search for a reliable and sufficiently selective circulating marker – corroborated in the present paper – biological assays have been employed with the hope of more meaningful results.

The ability of a histoscore to predict tumour response to antiangiogenic treatment, *e.g.* with bevacizumab, in this respect appears questionable according to the findings of our group (12). However, promising results were obtained with vascular tube formation assessment as a closer biological model: Vascular tube length showed a very pronounced and selective dependency on *in vitro* bevacizumab response (11), albeit in a very small sample of only 15 patients, all of whom had recurrent disease.

Vascular tube formation has recently shown to be promoted via Notch1 signalling pathways that mediate the epithelial to mesenchymal transition (EMT) during cervical cancer development (39), and its susceptibility to bevacizumab treatment suggested by the aforementioned study of our group was recently confirmed in ocular melanoma cells (40).

Other biological assays for development and spread of cervical cancer are under investigation, *e.g.* apoptosis induction in Jurkat cells (41), but none have been developed to a point of clinical application, and therefore clinical confirmation is notably absent.

Basically, the employment of biological models rather than the analysis of certain factors can be seen as a concession to the immense complexity of cervical cancer development and progression involving several stages that are meanwhile reasonably understood (42); this does not, however, change the fact that a plethora of molecules and pathways are involved in those stages, and that the biology of treatment response is an equally complicated matter.

Therefore, for the time being, biological modelling appears to be the most promising, albeit demanding, approach to prognosis assessment and treatment selection in cervical cancer. Apparently, the closer a biological model comes to the actual process of tumour angiogenesis, the more accurately it predicts bevacizumab treatment success. This concept, being basically plausible, is rather speculative at the moment and needs to be proven (or disproven) by further research.

Consequently, the development of a tool to identify the subset of patients, who will benefit from antiangiogenic treatment modalities, is in principle possible, but the circulating angiogenic factors examined in the present study most likely do not provide a sufficient basis for this. This reflects the rather complicated biological basis of tumour progression and regression under therapy, and the role of angiogenic factors within those processes; concentrations of circulating angiogenic factors before treatment are not inevitably coupled to those under conventional chemotherapy. Chemotherapy-induced tumour regression mobilises endothelial precursor cells (EPC) and their progenitors, which in turn can promote tumour neovascularisation (43), and this process – in addition to the angiogenesis involved in the primary growth of the tumour, which may or may not be reflected by circulating factors – is the very target of antiangiogenic modalities in combination with chemotherapy.

It is, therefore, unlikely that a single marker will turn out to be the 'magic wand' for the accomplishment of this undoubtedly crucial task. At the very least, a diagnostic index including a certain number of variables would be required; according to the research of our group, plasma VEGF (12) and endoglin (13) concentrations are likely candidates, especially since VEGF binding and subsequent inactivation is the key mechanism of bevacizumab action (14). However, despite the undisputable fact that these factors show a correlation with tumour biology and malignancy, our present appraisal of the selective potential of circulating angiogenic factors is sceptical. The involvement of an only partly understood multitude of molecules in the different stages of tumour development, progression and (treatment-induced) regression makes us lean more towards biological assays such as vascular tube formation (11) at present.

Clearly, the utility of this assay needs confirmation, because our results were obtained in a very small sample. However, considering the obvious demand for and the present lack of a reliable stratification method, and the particularly pronounced relationship between vascular tube length and *in vitro* bevacizumab response (11), further studies appear warranted. The required study design for a promising trial is rather straightforward, albeit elaborate. The most promising approach will be a randomized controlled trial of adjuvant bevacizumab treatment with complete assessment of relevant angiogenic factors and vascular tube formation assay, but without previous stratification of patients as a consequence. Such a study without the vascular tube formation assay is currently underway (14, 15), and if its results are conclusive, the involvement of vascular tube formation may become redundant. However, if this is not the case, vascular tube formation should be involved in a trial allowing for an assessment of the global impact of adjuvant antiangiogenic treatment modalities (regardless of their economical practicability); a better understanding of the independent prognostic role of individual angiogenic factors

and biological assays; a sound basis for the development of compound indices which might or might not allow the selection of 'patients at risk' for a benefit of antiangiogenic treatment; the mathematical modelling of the impact of the aforementioned selection.

The consequences of such a trial may be breaking new ground in the treatment of uterine cervix malignancies (20), and the development of strategies for patient selection will become all the more important.

Acknowledgements

Hartmut Buhck, M.D., provided editorial advice and assistance in statistical data evaluation as well as for the methodical aspects of result interpretation.

References

1 Hakim AA and Dinh TA: Worldwide impact of the human papillomavirus vaccine. Curr Treat Options Oncol *10*: 44-53, 2009.

2 Parkin DM, Bray F, Ferlay J and Pisani P: Global cancer statistics, 2002. CA Cancer J Clin *55*: 74-108, 2005.

3 Karimi Zarchi M, Behtash N, Chiti Z and Kargar S: Cervical cancer and HPV vaccines in developing countries. Asian Pac J Cancer Prev *10*: 969-974, 2009.

4 Corusić A, Skrgatić L, Mahovlić V, Mandić V, Planinić P and Karadza M: Cervical cancer as a public health issue--what next? Coll Antropol *34*: 301-307, 2010.

5 Statistisches Bundesamt: Todesursachen in Deutschland. Gestorbene in Deutschland an ausgewählten Todesursachen 2008. Fachserie 12, Reihe 4. Wiesbaden: Statistisches Bundesamt, 2010.

6 Statistisches Bundesamt: Todesursachen in Deutschland. Gestorbene in Deutschland an ausgewählten Todesursachen 2007. Fachserie 12, Reihe 4. Wiesbaden: Statistisches Bundesamt, 2009.

7 Horner MJ, Ries LAG, Krapcho M, Neyman N, Aminou R, Howlader N, Altekruse SF, Feuer EJ, Huang L, Mariotto A, Miller BA, Lewis DR, Eisner MP, Stinchcomb DG and Edwards B: SEER Cancer Statistics Review, 1975-2006, National Cancer Institute, Bethesda, MD,USA. http://seer.cancer.gov/csr/1975_2006/ based on November 2008 SEER data submission, posted to the SEER web site, 2009.

8 del Campo JM, Prat A, Gil-Moreno A, Perez J and Parera M: Update on novel therapeutic agents for cervical cancer. Gynecol Oncol *110*: S72-76, 2008.

9 Fujimoto J: Novel strategy of anti-angiogenic therapy for uterine cervical carcinomas. Anticancer Res *29*: 2665-2669, 2009.

10 Kim YH, Kim MA, Park IA, Park WY, Kim JW, Kim SC, Park NH, Song YS and Kang SB: VEGF polymorphisms in early cervical cancer susceptibility, angiogenesis, and survival. Gynecol Oncol *119(2)*: 232-236, 2010.

11 Landt S, Heidecke H, Korlach S, Reuter C, Schwidde I, Barinoff J, Schmid P, Sehouli J and Kümmel S: *In vitro* vascular tube formation testing as a tool for treatment individualisation in patients with cervical cancer. Anticancer Res *31*: 2609-2616, 2011.

12 Landt S, Heidecke H, Reuter C, Korlach S, Blohmer J-U, Lichtenegger W, Heusner T, Schmid P, Sehouli J, Thill M and Kümmel S: The utility of an *in vitro* angiogenesis score for prognosis assessment in patients with cervical cancer. Anticancer Res *31*: 2645-2650, 2011.

13 Landt S, Mordelt K, Schwidde I, Barinoff J, Korlach S, Sehouli J, Lichtenegger W and Kümmel S: Prognostic significance of the angiogenesis factors angiogenin, endoglin and endostatin in uterine cervical cancer. Anticancer Res *31*: 2651-2656, 2011.

14 Monk BJ, Willmott LJ and Sumner DA: Anti-angiogenesis agents in metastatic or recurrent cervical cancer. Gynecol Oncol *116*: 181-186, 2010.

15 Tewari KS and Monk BJ: Recent achievements and future developments in advanced and recurrent cervical cancer: trials of the Gynecologic Oncology Group. Semin Oncol *36*: 170-180, 2009.

16 Willmott LJ and Monk BJ: Cervical cancer therapy: current, future and anti-angiogenesis-targeted treatment. Expert Rev Anticancer Ther *9*: 895-903, 2009.

17 Randall LM, Monk BJ, Darcy KM, Tian C, Burger RA, Liao SY, Peters WA, Stock RJ and Fruehauf JP: Markers of angiogenesis in high-risk, early-stage cervical cancer: A Gynecologic Oncology Group study. Gynecol Oncol *112*: 583-589, 2009.

18 Zaghloul MS, El Naggar M, El Deeb A, Khaled H and Mokhtar N: Prognostic implication of apoptosis and angiogenesis in uterine cervical cancer. Int J Radiat Oncol Biol Phys *48*: 1409-1415, 2000.

19 Abulafia O, Triest WE and Sherer DM: Angiogenesis in malignancies of the female genital tract. Gynecol Oncol *72*: 220-231, 1999.

20 Moore DH: Chemotherapy for advanced, recurrent, and metastatic cervical cancer. J Natl Compr Canc Netw *6*: 53-57, 2008.

21 Takano M, Kikuchi Y, Kita T, Goto T, Yoshikawa T, Kato M, Watanabe A, Sasaki N, Miyamoto M, Inoue H and Ohbayashi M: Complete remission of metastatic and relapsed uterine cervical cancers using weekly administration of bevacizumab and paclitaxel/carboplatin. Onkologie *32*: 595-597, 2009.

22 Wright JD, Viviano D, Powell MA, Gibb RK, Mutch DG, Grigsby PW and Rader JS: Bevacizumab combination therapy in heavily pretreated, recurrent cervical cancer. Gynecol Oncol *103*: 489-493, 2006.

23 Monk BJ, Sill MW, Burger RA, Gray HJ, Buekers TE and Roman LD: Phase II trial of bevacizumab in the treatment of persistent or recurrent squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. J Clin Oncol *27*: 1069-1074, 2009.

24 Tan BR, McLeod HL: Pharmacogenetic influences on treatment response and toxicity in colorectal cancer. Semin Oncol *32*: 113-119, 2005.

25 Ebos JM, Lee CR and Kerbel RS: Tumor and host-mediated pathways of resistance and disease progression in response to antiangiogenic therapy. Clin Cancer Res *15*: 5020-5025, 2009.

26 Gressett SM and Shah SR: Intricacies of bevacizumab-induced toxicities and their management. Ann Pharmacother *43*: 490-501, 2009.

27 Randall LM and Monk BJ: Bevacizumab toxicities and their management in ovarian cancer. Gynecol Oncol *117*: 497-504, 2010.

28 Golshayan AR, Brick AJ and Choueiri TK: Predicting outcome to VEGF-targeted therapy in metastatic clear-cell renal cell carcinoma: data from recent studies. Future Oncol *4*: 85-92, 2008.
29 Hoyt K, Warram JM, Umphrey H, Belt L, Lockhart ME, Robbin ML and Zinn KR: Determination of breast cancer response to bevacizumab therapy using contrast-enhanced ultrasound and artificial neural networks. J Ultrasound Med *29*: 577-585, 2010.
30 Henriette Tanja L, Guchelaar HJ and Gelderblom H: Pharmacogenetics in chemotherapy of colorectal cancer. Best Pract Res Clin Gastroenterol *23*: 257-273, 2009.
31 Norum J, Nieder C and Kondo M: Sunitinib, sorafenib, temsirolimus or bevacizumab in the treatment of metastatic renal cell carcinoma: a review of health economic evaluations. J Chemother *22*: 75-82, 2010.
32 Zijlmans HJ, Fleuren GJ, Hazelbag S, Sier CF, Dreef EJ, Kenter GG and Gorter A: Expression of endoglin (CD105) in cervical cancer. Br J Cancer *100*: 1617-1626, 2009.
33 Minhajat R, Mori D, Yamasaki F, Sugita Y, Satoh T and Tokunaga O: Organ-specific endoglin (CD105) expression in the angiogenesis of human cancers. Pathol Int *56*: 717-723, 2006.
34 Carpini JD, Karam AK and Montgomery L: Vascular endothelial growth factor and its relationship to the prognosis and treatment of breast, ovarian, and cervical cancer. Angiogenesis *13*: 43-58, 2010.
35 Hawighorst H, Knapstein PG, Knopp MV, Weikel W, Brix G, Zuna I, Schonberg SO, Essig M, Vaupel P and van Kaick G: Uterine cervical carcinoma: comparison of standard and pharmacokinetic analysis of time-intensity curves for assessment of tumor angiogenesis and patient survival. Cancer Res *58*: 3598-3602, 1998.
36 Hong YS, Cho HJ, Kim SY, Jung KH, Park JW, Choi HS, Oh JH, Kim BC, Sohn DK, Kim DY and Chang HJ: Carbonic anhydrase 9 is a predictive marker of survival benefit from lower dose of bevacizumab in patients with previously treated metastatic colorectal cancer. BMC Cancer *9*: 246, 2009.
37 Pàez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F, Inoue M, Bergers G, Hanahan D and Casanovas O: Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell *15*: 220-231, 2009.
38 Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG and Kerbel RS: Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. Cancer Cell *15*: 232-239, 2009.
39 Srivastava S, Ramdass B, Nagarajan S, Rehman M, Mukherjee G and Krishna S: Notch1 regulates the functional contribution of RhoC to cervical carcinoma progression. Br J Cancer *102*: 196-205, 2010.
40 Yang H, Jager MJ and Grossniklaus HE: Bevacizumab suppression of establishment of micrometastases in experimental ocular melanoma. Invest Ophthalmol Vis Sci *51*: 2835-2842, 2010.
41 Aguilar-Lemarroy A, Romero-Ramos JE, Olimon-Andalon V, Hernandez-Flores G, Lerma-Diaz JM, Ortiz-Lazareno PC, Morgan-Villela G, Del Toro-Arreola S, Bravo-Cuellar A and Jave-Suarez LF: Apoptosis induction in Jurkat cells and sCD95 levels in women's sera are related to the risk of developing cervical cancer. BMC Cancer *8*: 99, 2008.
42 Kisseljov F, Sakharova O and Kondratjeva T: Cellular and molecular biological aspects of cervical intraepithelial neoplasia. Int Rev Cell Mol Biol *271*: 35-95, 2008.
43 Fürstenberger G, von Moos R, Lucas R, Thürlimann B, Senn HJ, Hamacher J and Boneberg EM: Circulating endothelial cells and angiogenic serum factors during neoadjuvant chemotherapy of primary breast cancer. Br J Cancer *94*: 524-531, 2006.

Received March 16, 2011
Revised June 20, 2011
Accepted June 21, 2011