A new angiogenesis prognostic index with VEGFA, PlGF, and angiopoietin 1 predicts survival in patients with advanced gastric cancer

Sedef Hande AKTAŞ[1], Hakan AKBULUT[1,]*, Ozan YAZICI[2], Nurullah ZENGİN[2],
Halime Nalan AKGÜN[1], Zeki ÜSTÜNER[3], Fikri İÇLİ[1]

[1]Department of Medical Oncology, Faculty of Medicine, Ankara University, Ankara, Turkey
[2]Department of Medical Oncology, Numune Hospital, Ankara, Turkey
[3]Department of Medical Oncology, Faculty of Medicine, Osmangazi University, Eskişehir, Turkey

Background/aim: The role of angiogenic factors in gastric cancer is not clear. We aimed to assess the role of vascular endothelial growth factor A (VEGFA), angiopoietin 1 (Ang-1), and placental growth factor (PlGF) in the prognosis of patients with advanced gastric cancer.

Materials and methods: Thirty consecutive patients treated with a modified DCF (docetaxel, cisplatin, and fluorouracil) regimen were included in the study. The plasma VEGFA, Ang-1, and PlGF levels of the patients before treatment and following two cycles of chemotherapy were measured and evaluated as prognostic factors.

Results: Poor performance status and lower Ang-1 levels were correlated with poor overall survival (OS). No significant correlation between VEGFA or PlGF and OS was found. An angiogenesis prognostic index (API) based on the levels of VEGFA, Ang-1, and PlGF was found to be highly correlated with OS. Performance status and API were found as independent prognostic factors for OS. Furthermore, a decrease in VEGFA by 25% from the pretreatment level was also found as a prognostic factor for OS independent of response to DCF regimen.

Conclusion: Our results support the use of the new API including VEGFA, Ang-1, and PlGF levels in patients with advanced gastric cancer as a predictor of survival.

Key words: Docetaxel, cisplatin, 5-fluorouracil, VEGFA, angiopoietin 1, placental growth factor, angiogenesis prognostic index, gastric cancer

1. Introduction

Angiogenesis is a crucial step in tumor progression and metastasis. Vascular endothelial growth factor A (VEGFA) and the VEGF pathway play a critical role in the growth of new vessels. Agents targeting angiogenesis, especially VEGFA and the VEGF pathway, have been the focus of research on angiogenesis-targeted therapies in cancer for the last 2 decades. The monoclonal antibodies binding VEGFA or small molecules inhibiting VEGF receptor tyrosine kinases have improved the treatment outcomes in a variety of solid tumors (1).

The prognosis of advanced gastric cancer patients is still poor. Although targeted therapies including trastuzumab in HER-2-expressing patients and bevacizumab targeting VEGF have been reported to improve treatment outcomes, the median overall survival (OS) time is still around 10 months (2). The DCF regimen including docetaxel, cisplatin, and 5-fluorouracil is one of the standard chemotherapy regimens yielding a median OS of 9.2 months (3). Because of the increased toxicity of the DCF regimen at standard doses, lowered doses of the drugs have also been implemented in many countries without using prophylactic colony-stimulating factors to decrease hematologic toxicities (4).

The predictive and prognostic role of angiogenic factors has also been studied widely in a variety of tumors. Though a majority of the studies on patients with operable solid tumors, including gastric cancer, have reported a significant correlation between the serum/plasma level of VEGFA and prognosis, this matter is not clear (5–8). Baseline VEGFA levels have also been suggested as a potential predictive marker for VEGF-pathway-targeted therapies (9). Early reduction of VEGFA levels in a small group of patients with gastric or gastroesophageal junction adenocarcinoma treated with cetuximab (a monoclonal antibody against epidermal growth factor receptor) and

chemotherapy including docetaxel and cisplatin has been reported as a predictive marker (10).

Placental growth factor (PlGF) is an angiogenic protein and involved in vascular development and maintenance. It has been shown to enhance cancer cell motility by mobilizing ERK1/2 phosphorylation and cytoskeletal rearrangement (11). The increased expression of PlGF in tumor tissues and elevated plasma levels have also been reported to be related with poor prognosis in various tumors including lung, colon, and gastric cancer (12–14).

The angiopoietin 1 (Ang-1)/Tie2 signaling pathway usually yields a stable vasculature with decreased permeability by enhancing the interaction between perivascular cells and endothelial cells (15). Though this interaction contributes to neovascularization, it usually limits tumor growth. Therefore, drugs targeting the Ang-1/Tie2 pathway are now under clinical trial. Although the increased expression of Ang-1 in gastric cancer has been reported as an indicator of advanced stages of gastric cancer (16), conflicting results regarding the better survival of patients with higher serum levels of Ang-1 have been reported in various cancers (17).

Although there are very few relevant trials, the combination of angiogenic factors seems to be a more useful prognostic parameter than the factors alone in cancer (5,18). Therefore, in the current study, we aimed to investigate the prognostic and predictive role of plasma VEGFA, PlGF, and Ang-1 levels and to describe a prognostic angiogenesis index based on the pretreatment levels of those angiogenic factors in patients with advanced gastric cancer treated with the DCF chemotherapy regimen.

2. Materials and methods

2.1. Patients and treatment

Patients who had advanced gastric carcinoma with measurable disease, were chemonaïve and planned to receive a modified DCF regimen (mDCF) only, were aged between 18 and 70 years, had ECOG performance status (PS) of 2 or less, and had adequate bone marrow reserve and normal renal functions were eligible for the study. The chemotherapy regimen was as follows: docetaxel (60 mg/m^2 on day 1), cisplatin (60 mg/m^2 on day 3), and 5-fluorouracil (600 mg/m^2 on days 1–4). Prophylactic granulocyte-colony stimulating factor was not routinely used.

Response to study treatment was assessed according to WHO criteria. Overall survival was accepted as the time interval between the first day of study treatment and date of death or last visit. Physical examination, complete blood count, liver and renal function tests, chest X-ray, and abdominal computerized tomography (CT) were carried out before the first cycle of DCF treatment. Thorax CT was carried out only if lung metastasis was suspected from the chest X-ray. While physical examination and routine blood tests were repeated before each cycle of mDCF, CT was repeated every 2 cycles of the treatment.

After receiving informed consent from the patients to participate in the trial, venous blood samples were taken on the day before the first cycle of chemotherapy and on the day of the response evaluation after 2 cycles of chemotherapy.

2.2. VEGFA, PlGF, and Ang-1 assays

Plasma VEGFA levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol (Human VEGF ELISA kit; BioSource, Cat No: KHG0111, Life Technologies, Grand Island, NY, USA). All the samples were assayed in duplicate. Briefly, plasma samples of 100 μL were added to the precoated wells and incubated for 2 h. Following the wash steps, 100 μL of Hu VEGF biotin conjugate was added. After 1 h of incubation, the wells were washed three times and 100 μL of streptavidin-horseradish peroxidase (HRP) was added. Following 30 min of incubation, the reaction was stopped with 100 μL of stop solution and the absorbance was determined at 450 nm with a microplate reader. The VEGFA levels of the patients were corrected according to platelet levels (per 100,000 platelets). We used the corrected levels to disregard the possible VEGFA derived from platelets (19).

Plasma PlGF levels were assayed using a sandwich ELISA kit (DRG Diagnostics GmBH, Cat No: EIA-4529, Marburg, Germany). Briefly, 25 μL of plasma samples, controls, and standards plus 250 μL of dilution buffer were added to the wells precoated with a monoclonal antibody directed towards a unique antigenic site on the PlGF molecule and incubated for 30 min. After a washing cycle, 100 μL of a biotin-linked polyclonal antibody specific for PlGF was added to the wells and incubated for 60 min. After a second washing cycle, 100 μL of streptavidin/horseradish peroxidase conjugate was added to the wells and incubated for 30 min. Following a third washing cycle, 100 μL of substrate solution was added. After 30 min of incubation, the reaction was stopped by 100 μL of stop solution and the absorbance was quantitated at 450 nm with a microplate reader.

We measured the plasma Ang-1 levels using a sandwich ELISA kit (Ang-1 assay: USCN Life Sciences Inc., Cat No: SEA008Hu, Cologne, Germany). Briefly, 100 μL of plasma samples and standards were added to the wells precoated with a monoclonal antibody specific to human Ang-1 and incubated for 1 h. Following the wash steps, 100 μL of biotin-conjugated antibody specific to Ang-1 was added and incubated for 1 h. Following a washing cycle, 100 μL of avidin conjugated to HRP was added to each well and wells were incubated for 30 min. After a washing cycle, 90 μL of TMB substrate solution was added and incubated 15–20 min at 37 °C. The reaction was stopped with 50 μL

of stop solution and the absorbance was determined at 450 nm with a microplate reader.

2.3. Statistical analysis

Survival analyses were done according to the Kaplan–Meier method, and the log-rank test was used for survival comparisons. Cox's regression analysis was used to assess the significant predictors for survival. Age, sex, PS, histologic type, disease involvement sites, angiogenic growth factor levels, and changes by treatment were used as variables for the evaluation of response to treatment and overall survival. In order to find the cut-off values, receiver operating characteristic (ROC) curves, the area under the ROC curve (AUC), and their 95% confidence intervals (95% CIs) were calculated. The ROC curve for each factor was used to choose the optimal cut-off value with maximized sensitivity and specificity.

3. Results

3.1. Patient characteristics and the efficacy of the treatment

A total of 30 consecutive patients with advanced gastric cancer were included in the study. The patient characteristics are outlined in Table 1. All the patients were given a modified DCF regimen. The relative dose intensity was 90%. A total of 116 cycles were administered (median 3 cycles per patient, range: 2–8), and 40% of the patients were given second-line treatment. The second-line treatment regimens were FOLFIRI (fluorouracil, folinic acid, and irinotecan) for 4 patients, FEP (fluorouracil, epirubicin, and cisplatin) for 4 patients, capecitabine only for 2 patients, and PF (weekly fluorouracil plus paclitaxel) for 2 patients.

No complete response (CR) was achieved. There were 6 patients with partial response (PR). The objective response rate was 20%. The disease control rate including PR, minimal response, and stable disease was 66.7%, and 33.3% of the patients had progressive disease while on treatment. There was no significant correlation between response to chemotherapy and the studied parameters (tumor histology, disease involvement sites, sex, and PS, PlGF, Ang-1, and VEGFA levels).

Median OS time was 9.0 ± 1.5 months (95% CI: 6.0-11.9). The 1-year and 2-year OS rates were 46.7% and 20%, respectively (Figure 1).

The mDCF regimen was well tolerated. Only 11 patients (36.7%) had grade 3/4 toxicity. There were 5 cases of febrile neutropenia, 4 cases of grade 3 diarrhea, and 2 cases of grade 3 fatigue. One patient had sagittal sinus thrombosis 15 days after the second cycle of the mDCF. She completed the remaining 4 cycles of the first-line treatment without cisplatin.

Table 1. Patients characteristics.

Parameter	N
Median age (range)	54 (30–70)
Sex, female/male	16/14

Histologic types
    Signet-cell carcinoma
    Adenocarcinoma w/o signet cells
	
	10
	20

Disease involvement sites
    Peritoneal involvement w/o distant metastasis
    Distant metastasis (liver, lung, bone)
	
	20
	10 (10, 1, 1)

Performance status (ECOG)
    0
    1
    2
    3
	
	3
	20
	6
	1

The levels of angiopoietic factors (pg/mL), mean ± standard error of mean
    VEGFA (pg/mL)$^{\Sigma}$
    PlGF (pg/mL)
    Ang-1 (pg/mL)
	
	23.1 ± 5.1 [¥]; 29.7 ± 6.7 [*]
	20.8 ± 1.5 [¥]
	301.9 ± 74.7 [¥]

VEGFA: Vascular endothelial growth factor A, PlGF: placental growth factor, Ang-1: angiopoietin 1. [Σ]: VEGFA levels were corrected per 100,000 platelets. [¥]: Pretreatment levels, [*]: levels after 2 cycles of chemotherapy.
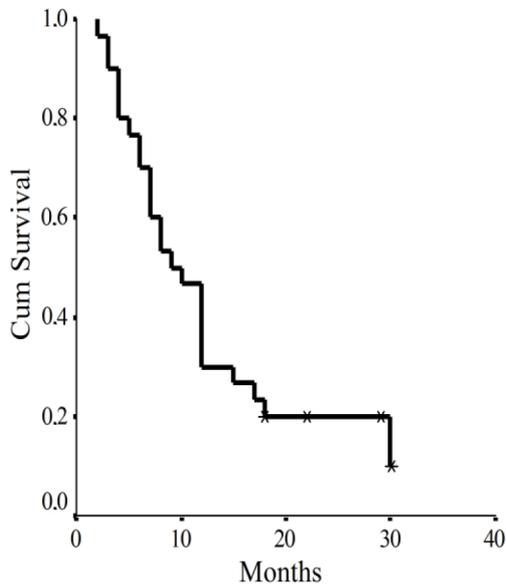
Figure 1. The OS curve of the entire cohort. The median OS time was 9 months and the 1-year and 2-year OS rates were 46.7% and 20%, respectively.
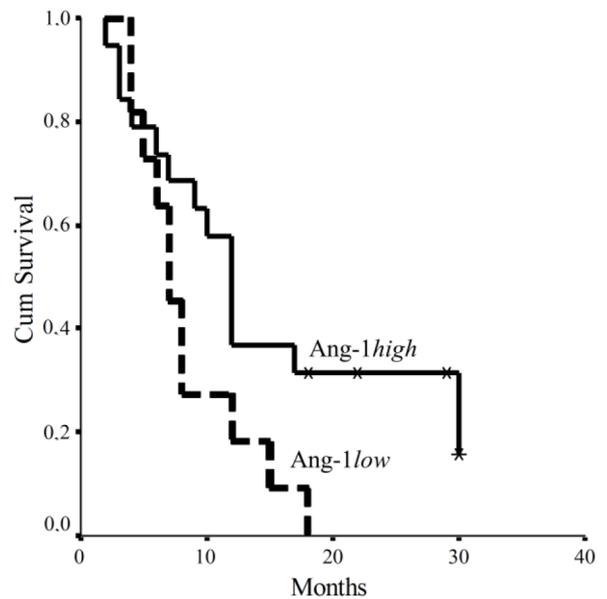

Figure 2. The OS curves according to the pretreatment Ang-1 levels (Ang-1*low*: patients with pretreatment Ang-1 levels lower than the cut-off value; Ang-1*high*: higher than the cut-off value). The median OS time was significantly higher in patients with low levels of Ang-1 (12.0 vs. 7.0 months; P = 0.049). Likewise, patients with low Ang-1 levels had better 2-year survival rates than the patients with high levels of Ang-1 (31.1% vs. 0%; P = 0.054).

3.2. Pretreatment Ang-1 levels and angiogenesis prognostic index correlated with survival in patients with gastric cancer

While the mean (±SEM) VEGFA levels (per 100,000 platelets) were 23.1 ± 5.1 pg/mL, the PlGF levels 20.8 ± 1.5 pg/mL and Ang-1 levels were 301.9 ± 74.7 pg/mL. The mean levels of VEGFA, PlGF, and Ang-1 were not significantly different between the patients with distant metastases and with peritoneal involvement. The cut-off values with maximized sensitivity and specificity from ROC curves were 43.7 pg/mL for VEGFA, 20.2 pg/mL for PlGF, and 273.4 pg/mL for Ang-1. Levels over the cut-off value were designated as *high* and levels below as *low*. Likewise, a decrease in the level of VEGFA of more than 25% of the pretreatment value after the second cycle of mDCF was designated as significant.

No correlation was found between the response to treatment and pretreatment levels of the angiogenic factor levels used in the study (P > 0.05) or VEGFA changes after 2 cycles of chemotherapy (P = 0.452).

Though not significant, the median OS time of the patients with low pretreatment VEGFA levels was longer than that of those with high levels (12.0 ± 3.7 vs. 8.0 ± 1.3 months; P = 0.159). Univariate analysis yielded the ECOG performance status (12.0 ± 1.2 (9.7–14.3) for PS ≤ 1 vs. 5.0 ± 1.3 (2.4–7.6) for PS = 2; P = 0.001) and Ang-1 levels (7.0 ± 0.8 for Ang-1_*low* vs. 12.0 ± 1.0 for Ang-1_*high*; P = 0.049) as the significant factors for OS (Figure 2). PlGF had no effect on survival of the patients (P = 0.787). The

median OS of the patients with a decrease in VEGFA levels following 2 cycles of chemotherapy was found to be longer than that of the patients with increased levels (10.0 ± 1.7 vs. 7.0 ± 2.1; HR: 0.37 (0.14–0.93).

An angiogenesis prognostic index (API) based on the levels of plasma VEGFA, PlGF, and Ang-1 was established. One point was assigned for each level of the following:

- VEGFA_*high*
- PlGF_*high*
- Ang-1_*low*

While the patients with 0–1 points were designated as API_*low*, those with 2–3 points were designated as API_*high*. The patients with API_*low* had a significantly higher median OS time when compared to API_*high* ones (12.0 ± 2.2 (7.7–16.0) vs. 8.0 ± 1.5 (5.0–10.9); P = 0.028). Likewise, the 2-year survival rate of API_*low* patients was significantly higher than that of the API-*high* patients (38% vs. 5%; P = 0.013).

Multivariate analysis yielded good PS (0–1) and lower API (0–1) as independent prognostic factors for OS (Table 2).

Table 2. The results of the multivariate analysis.

Variables	Hazard ratio (95% CI)	P
PS (ECOG) (≤1 vs. >1)	0.145 (0.044–0.472)	0.001
Age	1.280 (0.498–3.287)	0.609
Disease involvement sites (peritoneal involvement vs. distant metastasis)	1.836 (0.731–4.608)	0.196
API (low vs. high)	0.264 (0.096–0.727)	0.010

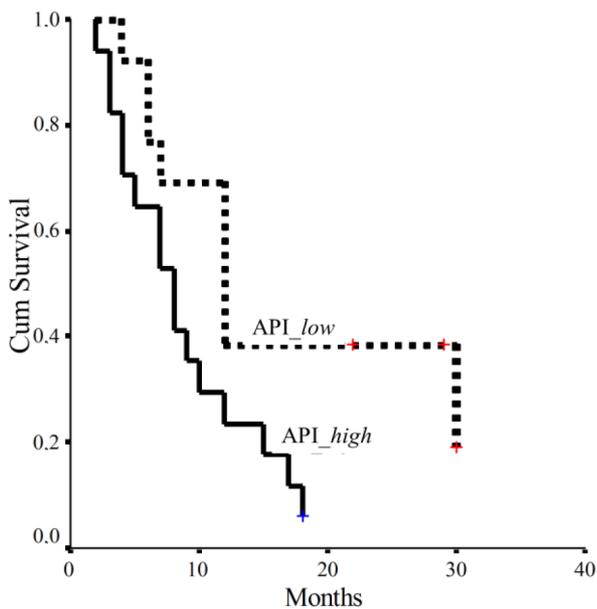PS: Performance status, API: angiogenesis prognostic index, CI: confidence interval.

Figure 3. The OS curves according to the angiogenesis prognostic index (API) (API_low patients with scores of 0–1; API_high with scores of 2–3). The median OS time and 2-year survival rates of the patients with API_low were significantly better than those of patients with API_high (12.0 vs. 8.0; P = 0.028 and 38% vs. 5%; P = 0.013, respectively).

4. Discussion

The poor prognosis of advanced gastric cancer has not changed substantially in the more than 2 decades since the implementation of platinum-based regimens (20). The treatment outcomes of our cohort of patients treated with a modified DCF regimen were quite similar to those of previous reports. Although the response rate is lower with the modified regimen in the current cohort when compared to the original report of Van Cutsem et al. with higher doses of DCF regimen (37% vs. 20%), the median OS times and 2-year survival rates are comparable (9 months vs. 9.2 months and 20% vs. 18%, respectively) (3). The disease stabilization rates in both trials are similar

(66.7% vs. 67%). Our results and other reports with the DCF regimen suggest that along with their cytotoxic effects on gastric cancer the drugs in this combination might change the behavior of the disease, possibly by inhibition of angiogenesis, to improve the survival (21,22).

Though angiogenesis plays a critical role in tumor growth and metastasis, the prognostic role of pro- and antiangiogenic factors in patients with gastric cancer is still controversial. The plasma levels of angiogenic factors including VEGFA, PlGF, and Ang-1 vary greatly in patients with advanced cancer (5,7,10,12,18). Although positive correlations have been reported with the levels of proangiogenic factors and the stage of various tumors, no consistent data exist with regard to the factor levels and the metastatic sites in advanced disease (5,8,10). Likewise, we could not find a significant difference between the VEGFA, PlGF, and Ang-1 levels of the patients with distant metastases and with peritoneal involvement.

Patients with VEGFA-positive gastric cancer have been reported to have significantly poorer prognosis than those with VEGFA-negative tumors (23). In a recent metaanalysis, it was shown that VEGFA expression by immunohistochemistry has an unfavorable impact on OS (24). Although reduction of serum VEGFA levels following the administration of cetuximab and combination chemotherapy has been found as a predictive marker, in vitro studies with 5-fluorouracil and cisplatin reported upregulation of VEGFA expression (25,26). Accordingly, correction of the serum levels of VEGFA by the number of platelets has been shown to correlate with the tumor expression of VEGFA (27). Likewise, in the current study, though not significant we found a trend that lower pretreatment VEGFA levels correlated with increased OS. Interestingly, we found that a 25% or more decrease in VEGFA level following 2 cycles of chemotherapy was significantly correlated with improved survival.

PlGF, a ligand for VEGFR-1 (Flt1), is known as a synergistic factor to VEGFA-mediated angiogenesis in cancer (28). PlGF stimulates the proliferation of endothelial cells and indirectly upregulates the expression

of various proangiogenic factors including VEGFA, FGF2, and PDGFB (29). Previous reports suggested that plasma PlGF levels are upregulated and correlated with survival in various cancers (12–14). Surprisingly, serum PlGF was found to be more useful than VEGFA in predicting worse prognostic features and survival in patients with operable gastric cancer (14) and hepatocellular cancer (30). However, in the current study, VEGFA seems to be more prognostic than PlGF in advanced disease. We could not find any significant relation between PlGF and survival in our cohort of patients.

Ang-1 promotes endothelial cell survival and maintains a quiescent vasculature via Tie-2 activation (31). The antagonistic activity of Ang-2 on Ang-1/Tie-2 signaling is suggested to balance Tie-2 signaling and regulates vascular homeostasis (32,33). In this context, the induced angiogenesis by Ang-2 in the presence of VEGFA expression suggests a possible inhibitory role of increased Ang-1 expression in the tumor microenvironment as part of an angiogenesis switch (34). Ang-1 is usually reported to be upregulated in different types of tumors and a correlation has been reported with the lymph node status and advanced stages (16). However, in many tumor models increased expression of Ang-1 has been found to be related to reduced tumor growth (35). Likewise, an increase in the serum level of Ang-1 with respect to Ang-2 in myeloma patients who responded to bortezomib treatment supports the antitumoral effects of this cytokine (36). However, the prognostic role of pretreatment Ang-1 levels in various cancers is contradictory (37,38). In the current study, we found that increased Ang-1 levels were significantly correlated with a favorable prognosis (Figure 2).

Because of the complex nature of angiogenesis, instead of using a certain factor alone, a combination of angiogenic factors might be a more reasonable biomarker. Previously we have shown that an index based on VEGFA, basic fibroblast growth factor (bFGF), and nitric oxide (NO) was a prognostic factor in patients with colorectal cancer (5). Likewise, in a recent study Park et al. found that an adjusted total value of four angiogenic factors (VEGFA, fibroblast growth factor 2 (FGF2), epidermal growth factor (EGF), and hepatocyte growth factor (HGF)) was better than any single factor (18). Accordingly, we established a simple API regarding the levels of VEGFA, PlGF, and Ang-1 in the current study. The proangiogenic factors of VEGFA and PlGF were selected for their tumor-promoting actions and Ang-1 for its mainly antitumoral properties (24,28,35). We found a low API score to be an important prognostic factor for patients with advanced gastric cancer (Table 2; Figure 2).

In conclusion, our results suggest that new parameters utilizing the combination of angiogenic factors could be useful as biomarkers in advanced gastric cancer. It is likely that those combined parameters would deserve further research for their ability to select patients who could benefit from antiangiogenesis treatment modalities. Likewise, a decrease in VEGFA levels following cancer treatment with either chemotherapy alone or combined with biologicals may also be used as a surrogate marker of survival in patients with gastric cancer.

References

1. Cook KM, Figg WD. Angiogenesis inhibitors: current strategies and future prospects. CA Cancer J Clin 2010; 60: 222-243.

2. Khokhar NZ, Jiang Y, Benson AB, Ajani JA, Mulcahy MF. Refining docetaxel-containing therapy for gastric cancer. Gastrointest Cancer Res 2011; 4: 96-105.

3. Van Cutsem E, Moiseyenko VM, Tjulandin S, Majlis A, Constenla M, Boni C, Rodrigues A, Fodor M, Chao Y, Voznyi E et al. Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. J Clin Oncol 2006; 24: 4991-4997.

4. Shah MA, Jhawer M, Ilson DH, Lefkowitz RA, Robinson E, Capanu M, Kelsen DP. Phase II study of modified docetaxel, cisplatin, and fluorouracil with bevacizumab in patients with metastatic gastroesophageal adenocarcinoma. J Clin Oncol 2011; 29: 868-874.

5. Akbulut H, Altuntas F, Akbulut KG, Ozturk G, Cindoruk M, Unal E, Icli F. Prognostic role of serum vascular endothelial growth factor, basic fibroblast growth factor and nitric oxide in patients with colorectal carcinoma. Cytokine 2002; 20: 184-190.

6. Jain RK, Duda DG, Willett CG, Sahani DV, Zhu AX, Loeffler JS, Bachelor TT, Sorensen AG. Biomarkers of response and resistance to antiangiogenic therapy. Nat Rev Clin Oncol 2009; 6: 327-338.

7. Burstein HJ, Chen YH, Parker LM, Savoie J, Younger J, Kuter I, Ryan PD, Garber JE, Chen H, Campos SM et al. VEGFA as a marker for outcome among advanced breast cancer patients receiving anti-VEGFA therapy with bevacizumab and vinorelbine chemotherapy. Clin Cancer Res 2008; 14: 7871-7877.

8. Al-Moundhri MS, Al-Shukaili A, Al-Nabhani M, Al-Bahrani B, Burney IA, Rizivi A, Ganguly SS. Measurement of circulating levels of VEGF-A, -C, and -D and their receptors, VEGFR-1 and -2 in gastric adenocarcinoma. World J Gastroenterol 2008; 14: 3879-3883.

9. Hanrahan EO, Ryan AJ, Mann H, Kennedy SJ, Langmuir P, Natale RB, Herbsty RS, Johnson BE, Heymach JV. Baseline vascular endothelial growth factor concentration as a potential predictive marker of benefit from vandetanib in non-small cell lung cancer. Clin Cancer Res 2009; 15: 3600-3609.

10. Di Fabio F, Pinto C, Bucca E, Giaquinta S, Fabricio AS, Mutri V, Michilin S, Rojas Llimpe FL, Funaioli C, Gion M et al. Predictive value of circulating VEGFA in patients with advanced gastric or gastroesophageal junction (GEJ) adenocarcinoma treated with cetuximab in combination with cisplatin and docetaxel (Italian phase II DOCETUX Study). J Clin Oncol 2008; 26 (May 20 Suppl.): 15605.

11. Taylor AP, Leon E, Goldenberg DM. Placental growth factor (PlGF) enhances breast cancer cell motility by mobilising ERK1/2 phosphorylation and cytoskeletal rearrangement. Brit J Cancer 2010; 103: 82-89.

12. Zhang L, Chen J, Ke Y, Mansel RE, Jiang WG. Expression of placenta growth factor (PlGF) in non-small cell lung cancer (NSCLC) and the clinical and prognostic significance. World J Surg Oncol 2005; 3: 68.

13. Wei SC, Liang JT, Tsao PN, Hsieh FJ, Yu SC, Wong JM. Preoperative serum placenta growth factor level is a prognostic biomarker in colorectal cancer. Dis Colon Rectum 2009; 52: 1630-1636.

14. Chen CN, Hsieh FJ, Cheng YM, Cheng WF, Su YN, Chang KJ, Lee PH. The significance of placenta growth factor in angiogenesis and clinical outcome of human gastric cancer. Cancer Lett 2004; 213: 73-82.

15. Brudno Y, Ennett-Shepard AB, Chen RR, Aizenberg M, Mooney DJ. Enhancing microvascular formation and vessel maturation through temporal control over multiple pro-angiogenic and pro-maturation factors. Biomaterials 2013; 34: 9201-9209.

16. Moon WS, Park HS, Yu KH, Jang KY, Kang MJ, Park H, Tarnawski AS. Expression of angiopoietin 1, 2 and their common receptor Tie2 in human gastric carcinoma: implication for angiogenesis. J Korean Med Sci 2006; 21: 272-278.

17. Fagiani E, Christofori G. Angiopoietins in angiogenesis. Cancer Lett 2013; 328: 18-26.

18. Park DJ, Yoon C, Thomas N, Ku GY, Janjigian YY, Kelsen DP, Ilson DH, Goodman KA, Tang LH, Strong VE et al. Prognostic significance of targetable angiogenic and growth factors in patients undergoing resection for gastric and gastroesophageal junction cancers. Ann Surg Oncol 2014; 21: 1130-1137.

19. George ML, Eccles SA, Tutton MG, Abulafi AM, Swift RI. Correlation of plasma and serum vascular endothelial growth factor levels with platelet count in colorectal cancer: clinical evidence of platelet scavenging? Clin Cancer Res 2000; 6: 3147-3152.

20. Wagner AD, Grothe W, Haerting J, Kleber G, Grothey A, Fleig WE. Chemotherapy in advanced gastric cancer: a systematic review and meta-analysis based on aggregate data. J Clin Oncol 2006; 24: 2903-2909.

21. Ajani JA. Optimizing docetaxel chemotherapy in patients with cancer of the gastric and gastroesophageal junction - evolution of the docetaxel, cisplatin, and 5-fluorouracil regimen. Cancer 2008; 113: 945-955.

22. Aktas SH, Akbulut H, Akgun N, Icli F. Low dose chemotherapeutic drugs without overt cytotoxic effects decrease the secretion of VEGFA by cultured human tumor cells: A tentative relationship between drug type and tumor cell type response. Cancer Biomark 2012; 12: 135-140.

23. Maeda K, Chung YS, Ogawa Y, Takatsuka S, Kang SM, Ogawa M, Sawada T, Sowa M. Prognostic value of vascular endothelial growth factor expression in gastric carcinoma. Cancer 1996; 77: 858-863.

24. Peng L, Zhan P, Zhou Y, Fang WJ, Zhao P, Zheng Y, Zu N. Prognostic significance of vascular endothelial growth factor immunohistochemical expression in gastric cancer: a meta-analysis. Mol Biol Rep 2012; 39: 9473-9484.

25. Tsuchida R, Das B, Yeger H, Koren G, Shibuya M, Thorner PS, Baruchel S, Malkin D. Cisplatin treatment increases survival and expansion of a highly tumorigenic side-population fraction by upregulating VEGFA/Flt1 autocrine signaling. Oncogene 2008; 27: 3923-3934.

26. Riedel F, Gotte K, Goessler U, Sadick H, Hormann K. Targeting chemotherapy-induced VEGFA up-regulation by VEGFA antisense oligonucleotides in HNSCC cell lines. Anticancer Res 2004; 24: 2179-2183.

27. Poon RTP, Lau CPY, Cheung ST, Yu WC, Fan ST. Quantitative correlation of serum levels and tumor expression of vascular endothelial growth factor in patients with hepatocellular carcinoma. Cancer Res 2003; 63: 3121-3126.

28. Carmeliet P, Moons L, Luttun A, Vincenti V, Compernolle V, De Mol M, Wu Y, Bono F, Devy L, Beck H et al. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. Nat Med 2001; 7: 575-583.

29. Kim KJ, Cho CS, Kim WU. Role of placenta growth factor in cancer and inflammation. Exp Mol Med 2012; 44: 10-19.

30. Ho MC, Chen CN, Lee H, Hsieh FJ, Shun CT, Chang CL, Lai YT, Lee PH. Placenta growth factor not vascular endothelial growth factor A or C can predict the early recurrence after radical resection of hepatocellular carcinoma. Cancer Lett 2007; 250: 237-249.

31. Yuan HT, Khankin EV, Karumanchi SA, Parikh SM. Angiopoietin 2 Is a partial agonist/antagonist of Tie2 signaling in the endothelium. Mol Cell Biol 2009; 29: 2011-2022.

32. Hanahan D. Signaling vascular morphogenesis and maintenance. Science 1997; 277: 48-50.

33.   Fagiani E, Lorentz P, Kopfstein L, Christofori G. Angiopoietin-1 and -2 exert antagonistic functions in tumor angiogenesis, yet both induce lymphangiogenesis. Cancer Res 2011; 71: 5717-5727.

34.   Scharpfenecker M, Fiedler U, Reiss Y, Augustin HG. The Tie-2 ligand angiopoietin-2 destabilizes quiescent endothelium through an internal autocrine loop mechanism. J Cell Sci 2005; 118: 771-780.

35.   Thomas M, Augustin HG. The role of the angiopoietins in vascular morphogenesis. Angiogenesis 2009; 12: 125-137.

36.   Anargyrou K, Terpos E, Vassilakopoulos TR, Pouli A, Sachanas S, Tzenou T, Masouridis S, Christoulas D, Angelopoulou MK, Dimitriadou EM et al. Normalization of the serum angiopoietin-1 to angiopoietin-2 ratio reflects response in refractory/resistant multiple myeloma patients treated with bortezomib. Hematologica 2008; 93: 451-454.

37.   Tabata C, Hirayama N, Tabata R, Yasumitsu A, Yamada S, Murakami A, Iida S, Tamura K, Fukuoka K, Kuribayashi K et al. A novel clinical role for angiopoietin-1 in malignant pleural mesothelioma. Eur Respir J 2010; 36: 1099-1105.

38.   Rahbari NN, Schmidt T, Falk CS, Hinz U, Herber M, Bork U, Büchler MW, Weitz J, Koch M. Expression and prognostic value of circulating angiogenic cytokines in pancreatic cancer. BMC Cancer 2011; 11.