Myelosuppression of Thrombocytes and Monocytes Is Associated with a Lack of Synergy between Chemotherapy and Anti-VEGF Treatment[1]

Patrick Starlinger, Philipp Brugger, Dominic Schauer, Silvia Sommerfeldt, Dietmar Tamandl, Irene Kuehrer, Sebastian F. Schoppmann, Michael Gnant and Christine Brostjan

Department of Surgery, Medical University of Vienna, General Hospital, Vienna, Austria

Abstract

PURPOSE: Chemotherapeutic agents that have shown improved patient outcome when combined with anti–vascular endothelial growth factor (VEGF) therapy were recently identified to induce the mobilization of proangiogenic Tie-2–expressing monocytes (TEMs) and endothelial progenitor cells (EPCs) by platelet release of stromal cell–derived factor 1α (SDF-1α). VEGF blockade was found to counteract cell mobilization. We aimed to determine why agents like gemcitabine do not elicit TEM and EPC recruitment and may therefore lack synergy with anti-VEGF therapy. *EXPERIMENTAL DESIGN:* Locally advanced pancreatic cancer patients ($n = 20$) were monitored during 16 weeks of neoadjuvant therapy. Treatment was based on gemcitabine with or without the addition of bevacizumab. Blood levels of proangiogenic cell populations and angiogenesis factors were determined in 2-week intervals. *RESULTS:* The lack of EPC mobilization during gemcitabine therapy was associated with severe thrombocytopenia and reduced SDF-1α blood concentrations. Furthermore, myelosuppression by gemcitabine correlated significantly with loss of TEMs. With respect to angiogenic factors stored and released by platelets, plasma levels of the angiogenesis inhibitor thrombospondin 1 (TSP-1) were selectively decreased and correlated significantly with thrombocytopenia in response to gemcitabine therapy. *CONCLUSIONS:* A thorough literature screen identified thrombocytopenia as a common feature of chemotherapeutic agents that lack synergy with anti-VEGF treatment. Our results on gemcitabine therapy indicate that myelosuppression (in particular, with respect to thrombocytes and monocytes) interferes with the mobilization of proangiogenic cell types targeted by bevacizumab and may further counteract antiangiogenic therapy by substantially reducing the angiogenesis inhibitor TSP-1.

Neoplasia (2011) 13, 419–427

Introduction

Because access to the systemic blood flow is essential for neoplastic growth and metastasis, the inhibition of vessel formation through antiangiogenic drugs has become an attractive target in cancer therapy [1,2]. In this context, bevacizumab, a neutralizing monoclonal antibody to proangiogenic vascular endothelial growth factor (VEGF), has shown benefit as single agent or in combination with standard chemotherapy in various types of cancers [3]. Comprehensive phase 3 trials have documented that patients with metastatic breast cancer, colorectal cancer, or non–small cell lung cancer (NSCLC) profit from the addition of bevacizumab to chemotherapy [4–6]. However, several studies failed to detect benefits in overall survival (OS), progression-free survival (PFS), or objective response rate. For example, patients with pancreatic cancer receiving antiangiogenic therapy with bevacizumab showed negligible therapeutic improvements [7–9].

Despite the general dependence of neoplastic growth on neovascularization, the variance in bevacizumab efficacy may arise from biologic differences among tumor types. Thus, angiogenesis in neoplastic entities "nonresponsive" to bevacizumab might be sustained by factors other than VEGF. A change in balance by the induction of proangiogenic mediators such as basic fibroblast growth factor (bFGF) and platelet-derived endothelial cell growth factor (PD-ECGF) or the down-regulation of angiogenesis inhibitors like thrombospondin 1 (TSP-1) might promote neovessel formation [10]. However, pancreatic cancer patients were found to exhibit increased VEGF serum and tissue levels, which correlated with advanced stage, postoperative recurrence, metastasis, and prognosis of these patients [11,12]. Furthermore, inhibition of VEGF potently suppressed pancreatic tumor growth in several preclinical models thus arguing for a central role of VEGF in pancreatic cancer [13,14].

In addition to differences in cancer biology, it has been proposed that the choice of chemotherapy might determine the efficacy of anti-VEGF treatment [15]. Of note, striking therapeutic improvements by bevacizumab coadministration were observed for combination therapy with paclitaxel in metastatic breast cancer, 5′-fluorouracil (5-FU) and irinotecan in colorectal cancer as well as for paclitaxel and carboplatin in NSCLC [4–6]. In contrast, the combination of bevacizumab with gemcitabine as applied for pancreatic cancer was of minimal benefit [8,9]. Shaked et al. [15] were the first to investigate in a preclinical model whether selected chemotherapeutics have a substantial impact on the effectiveness of anti-VEGF treatment. They found that compounds differed significantly in the mobilization of proangiogenic endothelial progenitor cells (EPCs) and Tie-2–expressing monocytes (TEMs). An increase in circulating EPCs and TEMs was induced within 4 to 24 hours of paclitaxel, docetaxel, or 5-FU administration and was reflected in enhanced tumor infiltration by EPCs and TEMs. In contrast, chemotherapeutics such as gemcitabine or doxorubicin had no promoting effect on EPC or TEM recruitment. Importantly, treatment with a VEGF receptor antibody potently blocked this recruitment thereby blunting the "proangiogenic adverse effect" of chemotherapy. As a result, the addition of anti-VEGF therapy to paclitaxel caused enhanced suppression of tumor growth, whereas the combination with gemcitabine did not improve antitumor activity.

EPC mobilization was found to be central to the efficacy of combination treatments and seemed to be mediated by the release of stromal cell–derived factor 1α (SDF-1α) in response to chemotherapy. Corresponding to the growing evidence that megakaryocytes and platelets are the major sources of SDF-1α [16,17], the increase in SDF-1α plasma levels after paclitaxel administration was accompanied by a decrease of intracellular SDF-1α stored in platelets. No substantial changes in SDF-1α levels were observed after gemcitabine treatment. Therefore, the authors concluded that paclitaxel (as opposed to gemcitabine) induces EPC mobilization by prompting platelets to release SDF-1α.

The conclusions of the preclinical study were further strengthened by clinical data. A significant increase of EPCs and circulating SDF-1α was found in cancer patients within 4 hours of a single dose of taxane-based chemotherapy, whereas gemcitabine, doxorubicin, or cisplatin failed to induce proangiogenic cell recruitment [15,18].

In view of these findings, we focused on the systemic effects of a "nonresponsive" chemotherapeutic compound to further explain the lack of EPC and TEM mobilization. In the context of a clinical trial, locally advanced pancreatic cancer patients received neoadjuvant gemcitabine treatment with or without bevacizumab coadministration. We closely monitored soluble angiogenic factors and angiogenesis associated cell populations to obtain a detailed picture of the effects on the angiogenic balance by the chemotherapeutic regimen that had shown unfavorable outcome in combination with bevacizumab. Intriguingly, the myelosuppressive effect of gemcitabine resulting in thrombocytopenia correlated with severe changes in angiogenic factors, which may explain the failure of the combined anti-VEGF therapy.

Materials and Methods

Study Design and Patient Collective

Twenty previously untreated patients with locally advanced non-metastatic pancreatic cancer (T4, stage III) were enrolled in the study. Exclusion criteria comprised stage IA to IIB and stage IV disease, any previous systemic cancer treatment, major surgery within the last 28 days, a history of bleeding or coagulation disorders, as well as other malignant diseases within the last 5 years.

Patients were randomly assigned to two treatment arms. Both groups received 1000 mg/m² gemcitabine on days 1, 8, and 15 of four consecutive 4-week cycles. Group 1 (four women and five men of median age 65 years, ranging from 43 to 77 years) started with the biweekly addition of 5 mg/kg bevacizumab in week 3 of the second cycle. Patients in group 2 (five women and six men of median age 62 years, ranging from 52 to 80 years) received bevacizumab from the beginning of chemotherapy. The fourth cycle did not include bevacizumab, providing a gap of at least 8 weeks between the last antibody dose and pancreatic surgery. Blood samples for monitoring angiogenesis parameters were drawn from patients at 2-week intervals during the entire neoadjuvant treatment period. CA 19-9 tumor marker levels were determined by routine hospital analysis.

The clinical study was registered in the public trial registry EudraCT (no. 2005-004519-32). The study protocol and the analysis of blood samples were approved by the institutional ethics committee; all patients gave written informed consent.

Quantification of Angiogenic Factors

Blood was drawn into prechilled tubes containing citrate, theophylline, adenosine, and dipyridamole; was immediately placed on ice; and further processed within 30 minutes. After an initial centrifugation step at $1000g$ and 4°C for 10 minutes, the plasma supernatant was subjected to further centrifugation at $10,000g$ and 4°C for 10 minutes (to remove remaining platelets). Plasma samples were analyzed by enzyme-linked immunosorbent assay (ELISA) for concentrations of angiogenesis factors. Commercially available ELISA tests were applied for bFGF, TSP-1, SDF-1α (Quantikine; R&D Systems, Minneapolis, MN), and for VEGF-A (Quantikine; R&D Systems or Invitrogen Corp, Camarillo, CA), according to the manufacturers' instructions. A comparable "sandwich" ELISA system for PD-ECGF (detection range = 1–100 ng/ml) has previously been reported by us [19,20].

Quantification of EPCs and TEMs

Blood was drawn in EDTA tubes and subjected to staining with an appropriate set of antibodies for evaluation of TEMs (anti-human CD14-FITC, CD16-PC5, Tie2-PE) and EPCs (anti-human CD3-PC5, CD19-PC5, CD33-PC5, CD31-FITC, CD34-ECD, CD133-PE, and the viability stain 7-aminoactinomycin D). Following a "lyse-no-wash"

procedure with VersaLyse reagent (Immunotech, Beckman Coulter, Brea, CA) samples were analyzed with an FC500 flow cytometer (Beckman Coulter) for detection of viable endothelial progenitors (CD3$^-$, CD19$^-$, CD33$^-$, CD34$^+$, CD133$^+$) and TEMs (CD14$^+$, CD16$^+$, Tie2$^+$).

Evaluation of Response

Objective tumor response to neoadjuvant treatment was evaluated by computed tomography or magnetic resonance imaging and was defined in categories of progressive disease, stable disease, or partial remission according to Response Evaluation Criteria in Solid Tumors (RECIST). OS of patients was assessed in a 2-year follow-up period (with one patient alive at the time of study closure).

Statistical Analysis

Statistical analyses were carried out with SPSS 17.0.1 Software (SPSS, Inc, Chicago, IL) and were based on nonparametric tests (Spearman, Mann-Whitney U, and Wilcoxon test). Data are generally presented by box plot illustration; outliers and extreme values are not depicted.

Results

Bevacizumab Induces VEGF Feedback Production but Does Not Enhance Tumor Marker Reduction in Response to Chemotherapy

From August 2006 to September 2008, 20 patients with locally advanced pancreatic cancer were randomized into two treatment arms. Both study groups received neoadjuvant gemcitabine therapy on days 1, 8, and 15 of four consecutive 4-week cycles. Group 2 received biweekly addition of bevacizumab from treatment start, whereas group 1 had a delayed onset of bevacizumab therapy in week 3 of cycle 2. Blood samples were collected at 2-week intervals during the neoadjuvant treatment period to closely monitor therapy-induced changes in angiogenesis parameters (Figure 1A).

In accordance with previous reports [20,21], a rapid and significant ($P = .002$) increase of plasma VEGF levels was observed in response to the first bevacizumab administration, and VEGF values remained elevated for the subsequent treatment period (Figure 1, B and C). The "feedback production" of VEGF is known as a pharmacodynamic marker of bevacizumab therapy but does not counteract VEGF inactivation by the antibody [20,22]. Because group 1 started bevacizumab treatment with a delay of 6 weeks, the rise in VEGF plasma concentration was not detected before time point 5 of blood sampling. Correspondingly, a significant difference in VEGF blood levels between treatment groups was observed for the blood collection time points 2, 3, and 4 ($P = .042$, $P = .005$, and $P < .001$) when group 1 received single-agent gemcitabine.

To monitor tumor load in the course of neoadjuvant therapy, the tumor marker CA 19-9 was evaluated. A significant decline in CA 19-9 blood concentration ($P = .006$ for time points 1 and 8) was evident on treatment (Figure 1D). No significant difference between groups was observed when evaluated for the entire study period or for the first 6 weeks of gemcitabine therapy with or without bevacizumab (Figure 1E).

The potential of VEGF and CA 19-9 to predict therapy response or patient prognosis was further investigated. The baseline level or increase of VEGF on bevacizumab treatment (as determined after the first antibody administration or after completion of therapy) did not correlate with patient response according to RECIST criteria. Similarly, CA 19-9 pretreatment levels or therapy-associated changes did not differ significantly between responders and nonresponders. Furthermore, OS was not predicted by either parameter.

Gemcitabine Does Not Induce Significant EPC Mobilization throughout Therapy

Since Shaked et al. [15] reported a rapid (4 hours) mobilization of EPCs by paclitaxel as opposed to gemcitabine but did not investigate later time points of therapy, we monitored EPC blood counts by flow cytometry in 2-week intervals throughout neoadjuvant treatment (Figure 2, A and B). When EPC levels of sampling time points 2 to 8 were compared with baseline values, no significant change in EPC counts was observed. Furthermore, EPC measurements were assigned to two categories based on gemcitabine administration to more appropriately evaluate chemotherapy-induced effects. Median EPC values obtained by blood sampling 1 week after gemcitabine administration (time points 2, 4, 6, and 8) ranged at 1.5 cells/500,000 leukocytes and did not differ significantly from EPC counts recorded before therapy or after chemotherapy breaks (time points 1, 3, 5, and 7) with a median value of 2.0 cells/500,000 leukocytes. Importantly, EPC fluctuations were comparable between treatment groups and did not seem to be affected by the onset of anti-VEGF therapy (Figure 2B).

SDF-1α Release Is Counteracted by Thrombocytopenia

Platelet-released SDF-1α has been proposed to be the major inducer of EPC mobilization in response to chemotherapy [15]. Therefore, we investigated circulating SDF-1α values and their relation to platelet counts in seven patients of treatment group 1 and of group 2 during neoadjuvant gemcitabine treatment. Irrespective of treatment group, chemotherapy did not result in a significant elevation of SDF-1α plasma levels (median value of 1654 pg/ml after chemotherapy at time points 2, 4, 6, and 8 versus median value of 1729 pg/ml before therapy or after therapy breaks at time points 1, 3, 5, and 7). In contrast, SDF-1α levels showed a tendency to decline after gemcitabine administration (Figure 2C). Similarly, platelet counts were found to exhibit a pronounced decrease in response to gemcitabine treatment and to recover in intermitting periods (Figure 2D), with median values ranging at 171 G/L after chemotherapy versus 334 G/L before therapy or after therapy breaks ($P < .001$). To assess the potential impact of thrombocytopenia on the availability of circulating SDF-1α, plasma values of SDF-1α were adjusted to standard platelet counts of 300 G/L (Figure 2E). On the basis of the "normalized" SDF-1α levels, a significant induction of circulating SDF-1α values by gemcitabine treatment became apparent, which was followed by a decline to baseline levels in intermission periods ($P < .001$; value of 2879 pg/ml after chemotherapy versus value of 1085 pg/ml before therapy or after therapy breaks). Of note, no significant impact of bevacizumab addition and no difference between treatment groups were observed for SDF-1α or platelet fluctuations (data not shown).

Gemcitabine Reduces Circulating Monocytes and Proangiogenic TEMs

Because proangiogenic cell populations distinct from EPCs, such as TEMs, were shown to be induced by paclitaxel as opposed to gemcitabine and to be target of anti-VEGF therapy [15], we evaluated circulating TEM, total monocyte, and leukocyte counts during neoadjuvant treatment of pancreatic cancer patients. In accordance
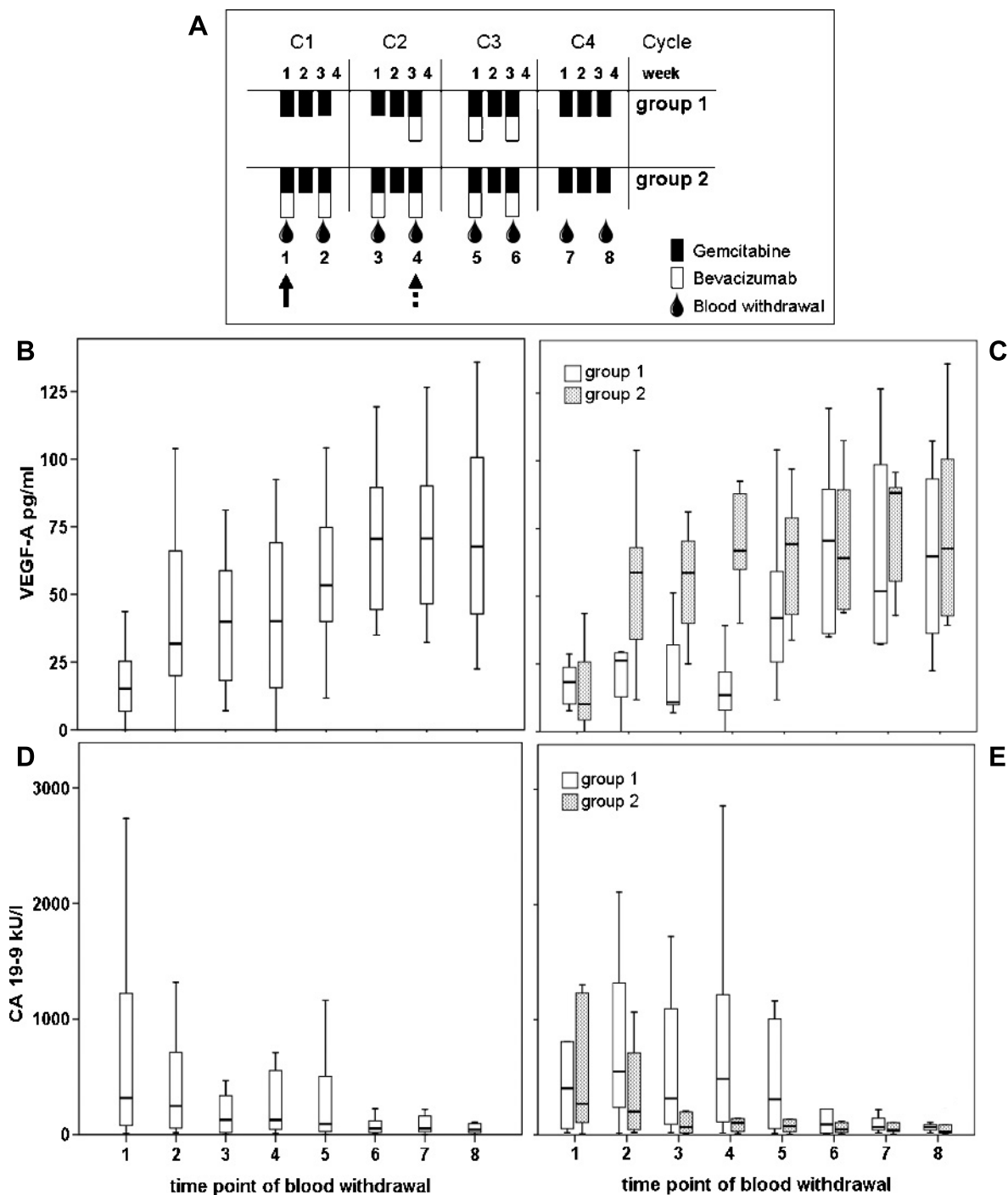
Figure 1. Blood levels of VEGF and CA 19-9 in pancreatic cancer patients undergoing neoadjuvant therapy. (A) Schematic representation of study design and blood sampling time points 1 to 8: The onset of bevacizumab administration is indicated by dashed and solid arrows for treatment groups 1 and 2, respectively. Plasma concentrations of VEGF (B, C) and CA 19-9 (D, E) according to blood collection schedule are illustrated by box plot for all patients $n = 20$ (B, D) or for the individual treatment groups $n_1 = 9$ and $n_2 = 11$ (C, E).

with the time course of platelets, leukocytes (Figure 3A), monocytes (Figure 3B), and TEMs (Figure 3C) also fluctuated corresponding to the chemotherapy schedule, which was reflected in a significant correlation ($P \leq .004$) between these cell populations and was indicative of a general myelosuppression.

Compared with the overall leukocyte population, the suppressive effect on blood monocytes was highly pronounced. Monocyte counts differed significantly ($P < .001$) between time points after gemcitabine

administration (median = 4.5×10^5 CD14$^+$ cells/ml blood) and time points before therapy or after recovery periods (median = 8.9×10^5 CD14$^+$ cells/ml blood). Similarly, TEM levels displayed a recurrent "drop-and-rebound" pattern and varied significantly with gemcitabine treatment schedule ($P = .002$; median value of 4273 CD14$^+$ Tie2$^+$ CD16$^+$ cells/ml blood after chemotherapy versus median value of 8865 CD14$^+$ Tie2$^+$ CD16$^+$ cells/ml blood before therapy or after therapy breaks). Comparable results were obtained for study arms 1 and 2,

and no significant impact of bevacizumab administration was recorded (data not shown).

Platelet-Derived Angiogenesis Inhibitor TSP-1 Is Reduced by Gemcitabine Therapy

Beside their central role in SDF-1α release and EPC recruitment, platelets abundantly store angiogenic factors [23,24]. As we had observed an intense reduction of platelet counts during chemotherapy, it was of interest to investigate whether thrombocytopenia was associated with a decrease in circulating angiogenic factors known to be released by platelets. In addition to VEGF (Figure 1, B and C), bFGF and PD-ECGF as well as TSP-1 were evaluated in patient plasma during neoadjuvant treatment (Figure 4, A–C). The proangiogenic molecules VEGF, bFGF, and PD-ECGF did not fluctuate in accordance with platelet counts. In contrast to VEGF, the plasma concentrations of bFGF and PD-ECGF did not vary with treatment group, that is, showed no bevacizumab-associated regulation. Of interest, blood levels of the potent antiangiogenic protein TSP-1 displayed a "drop-and-rebound" pattern reminiscent of platelet fluctua-

tions (Figure 4A), which was reflected in a significant correlation between parameters ($P < .001$, $k = 0.399$). Furthermore, TSP-1 levels differed significantly with treatment schedule ($P = .001$; median TSP-1 value of 43 ng/ml after chemotherapy versus median value of 65 ng/ml before therapy or after therapy breaks).

Discussion

The combination of chemotherapy with bevacizumab has shown varying outcome, ranging from a highly significant increase in OS to a complete lack of patient benefit when compared with chemotherapy without VEGF blockade [25]. This discrepancy may relate to tumor entity or combination of regimens. Indeed, increasing evidence argues in favor of the latter hypothesis. The currently conducted "RIBBON-2" trial on locally recurrent or metastatic breast cancer compares the efficacy of bevacizumab therapy in combination with distinct chemotherapeutic agents. Preliminary data presented at the 2010 ASCO Meeting [26] suggest that breast cancer patients receiving taxane or capecitabine profit from bevacizumab augmentation, whereas the
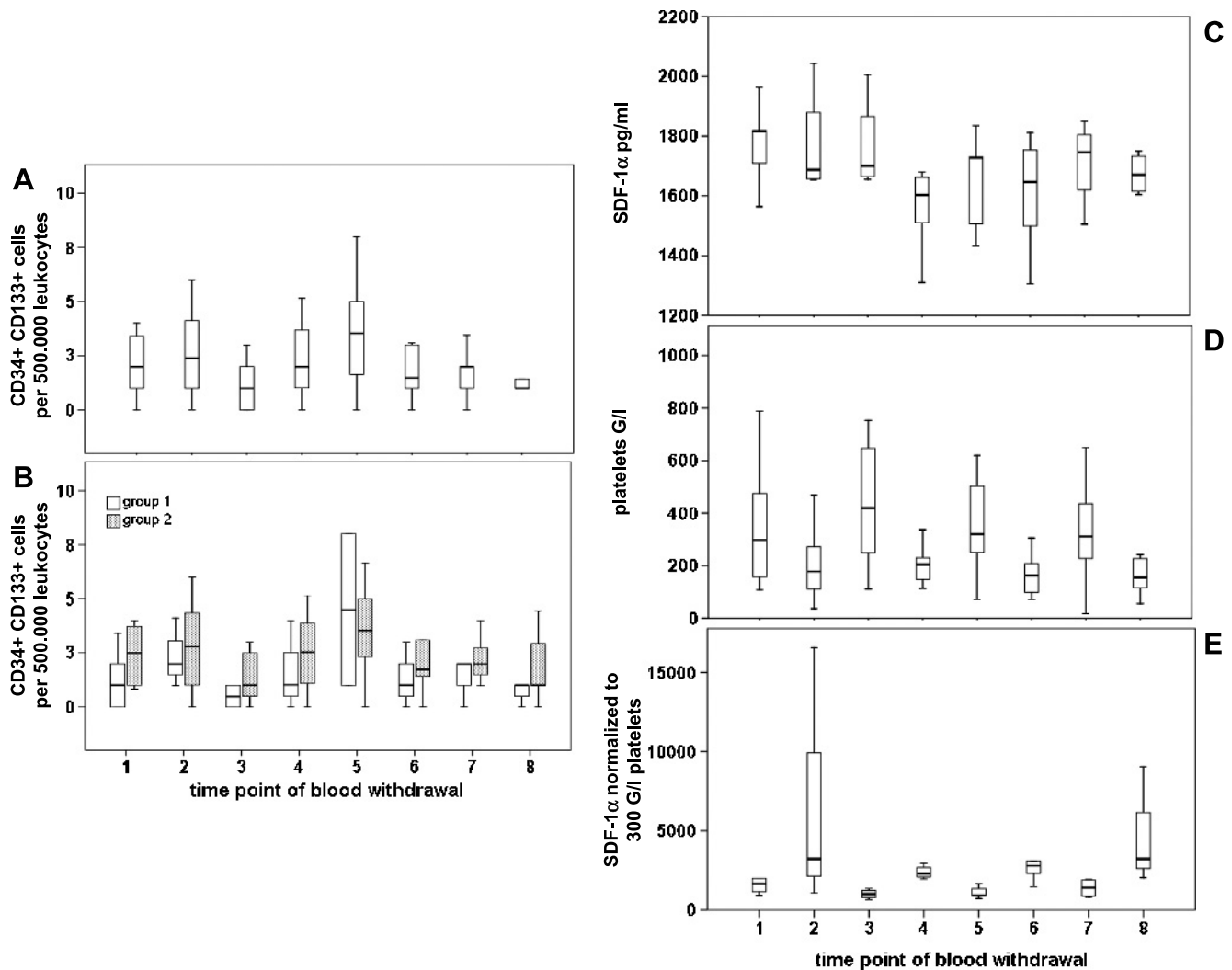

Figure 2. Effect of neoadjuvant treatment on EPC counts, blood platelets, and SDF-1α plasma levels of pancreatic cancer patients. Fluctuations of EPCs (CD34$^+$ CD133$^+$ blood cells) are presented by box plot for the entire study collective $n = 15$ (A) or separately for treatment arms $n_1 = 6$ and $n_2 = 9$ (B). C, D, and E illustrate the time course of SDF-1α ($n = 7$), blood platelets ($n = 19$), and SDF-1α levels normalized to a platelet count of 300 G/L ($n = 7$) irrespective of treatment group.
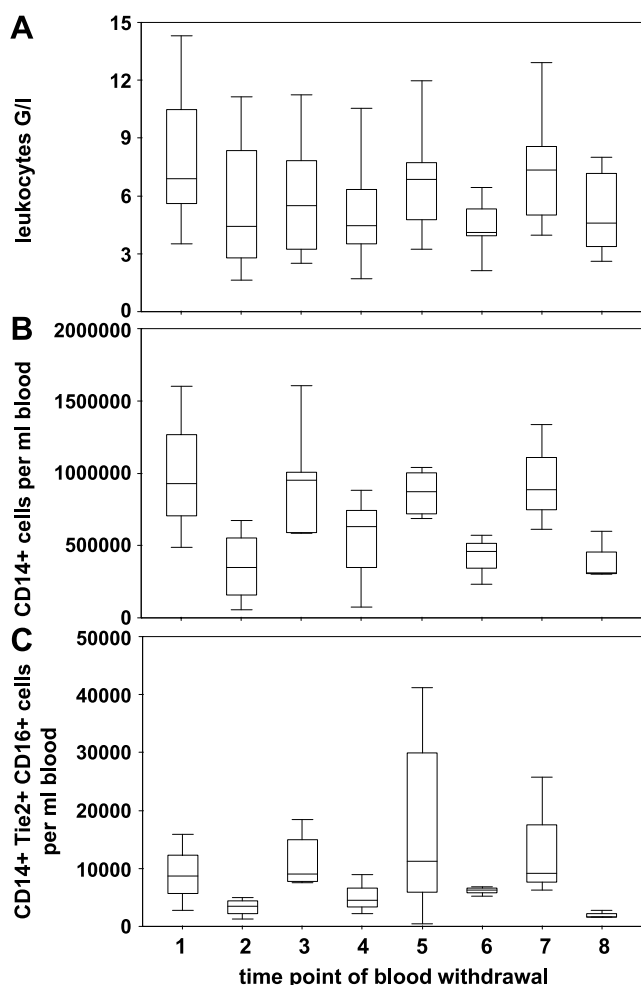
Figure 3. Changes in leukocyte, monocyte, and TEM counts during pancreatic cancer therapy. Total populations of blood leukocytes (A, $n = 17$), CD14$^+$ monocytes (B, $n = 7$), and the subset of CD14$^+$ CD16$^+$ Tie2$^+$ TEMs (C, $n = 7$) were monitored in patient blood by flow cytometry. Data are illustrated by box plot irrespective of treatment group.

istration documented the selective systemic response to VEGF inhibition, which has previously been reported and does not interfere with complete VEGF blockade [20,22]. However, the pharmacodynamic changes in circulating VEGF did not correlate with enhanced tumor marker decline (comparing treatment periods with and without bevacizumab supplementation) or with patient response and survival. Thus, the prolonged treatment with bevacizumab did not improve patient benefit in this clinical setting.

Importantly, we observed an intense and recurring myelosuppressive effect of gemcitabine treatment, which was reflected in blood components known to mediate proangiogenic cell mobilization. Platelet
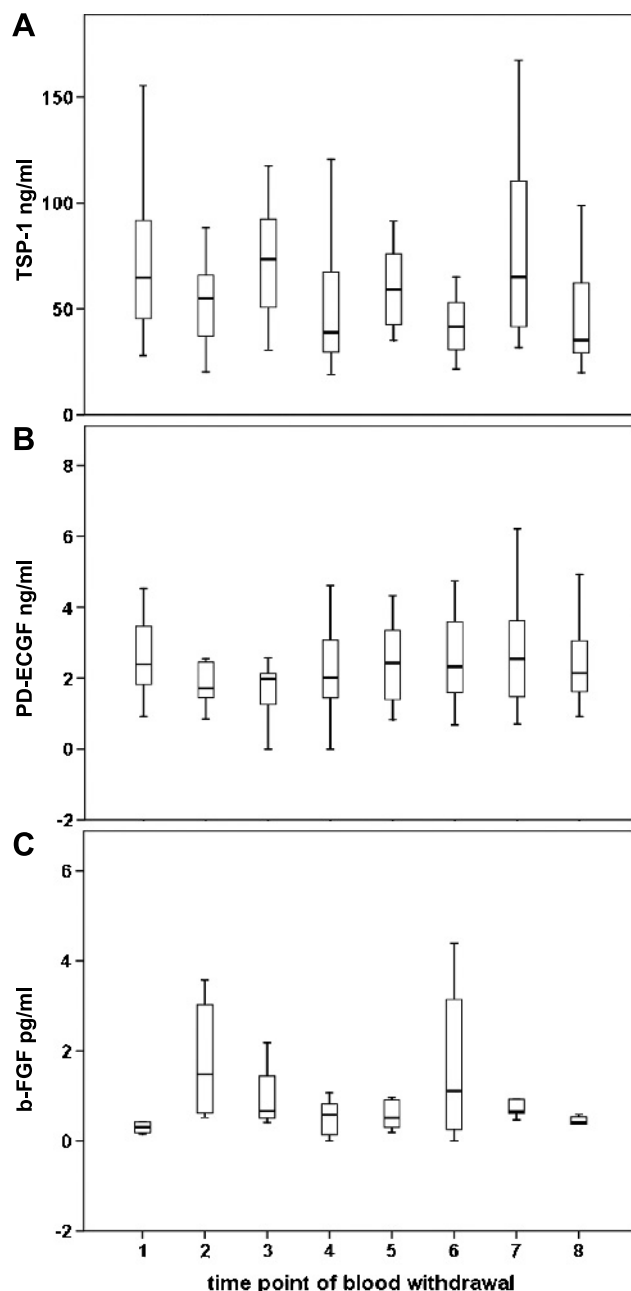
Figure 4. Effect of chemotherapy on circulating proangiogenic and antiangiogenic factors. The plasma levels of antiangiogenic TSP-1 (A, $n = 20$), proangiogenic PD-ECGF (B, $n = 20$), and bFGF (C, $n = 6$) were determined by ELISA and are presented by box plot in relation to blood sampling time points, irrespective of treatment group.

combination with gemcitabine seems unfavorable. This finding is supported by the observation that bevacizumab addition to gemcitabine therapy failed to achieve improvements for pancreatic cancer patients [9].

Shaked et al. [15] provided an intriguing explanation for the clinical results. They demonstrated in a mouse model that distinct therapeutics such as taxanes and 5-FU induce the rapid mobilization of proangiogenic cell populations (EPCs, TEMs) by platelet release of SDF-1α, whereas other regimens like gemcitabine or doxorubicin do not elicit this effect. Furthermore, they showed that VEGF blockade could counteract the proangiogenic cell recruitment thereby leading to selective treatment benefits for combinations with "mobilizing" agents. In contrast, "nonmobilizing" chemotherapeutics like gemcitabine lack synergism with anti-VEGF agents. We now propose that the distinct myelosuppressive properties of "nonmobilizing" regimens determine this lack of synergism.

In a clinical study on neoadjuvant chemotherapy, we investigated alterations in blood parameters of pancreatic cancer patients receiving continuous gemcitabine therapy and three or six doses of bevacizumab. The marked increase in VEGF plasma levels on bevacizumab admin-

counts were severely reduced in immediate response to gemcitabine administration and recovered in therapy breaks, thus yielding a characteristic "drop-and-rebound" pattern. In accordance with platelets being the major source for circulating SDF-1α, plasma levels of SDF-1α showed a tendency to decrease on gemcitabine treatment. Of interest, when SDF-1α values were adjusted for platelet counts, a remarkable and consistent increase in response to chemotherapy was observed, reminiscent of the SDF-1α release induced by "mobilizing" agents [15]. This suggests that gemcitabine may in fact trigger SDF-1α secretion by platelets resulting in an increased ratio of circulating SDF-1α to platelet counts. However, thrombocytopenia limits the available pool of SDF-1α, which results in a net decrease of SDF-1α blood levels. With SDF-1α being the central stimulus for chemotherapy-induced EPC mobilization [15], thrombocytopenia (and additional myelosuppression) may therefore prevent EPC recruitment. Correspondingly, we did not detect significant EPC induction during neoadjuvant treatment with gemcitabine.

Furthermore, myelosuppression was detectable in a remarkable decrease of monocytes and circulating proangiogenic TEMs. Because anti-VEGF therapy was also shown to block TEM recruitment by "mobilizing" agents [15], TEM reduction by myelosuppression may further account for the lack of synergism between "nonmobilizing" therapeutics and bevacizumab. Figure 5 illustrates the accordance of parameters involved in proangiogenic cell mobilization, which are affected by the myelosuppressive activity of gemcitabine.

In contrast to the pronounced effect on thrombocyte and monocyte counts, gemcitabine showed only a moderate suppression of total leukocytes in our study. Considering that neutrophils constitute most blood leukocytes, suppression of platelet and monocyte populations rather than neutropenia may thus be characteristic for "nonmobilizing" therapeutics. In support of this notion, the taxanes paclitaxel and docetaxel were shown to induce severe neutropenia but little toxicity to blood platelets [27] and have demonstrated "mobilizing" potential resulting in synergy with bevacizumab therapy [15]. Interestingly, two studies with cancer patients report on stable or even increasing monocyte counts in taxane combination therapies with substantial neutropenia [28,29]. Similarly, the "mobilizing" agent 5-FU does not affect monocyte counts in chemoradiotherapy [30] despite its potential to reduce blood neutrophils [31]. Thus, we propose that the distinct capacity of chemotherapeutics to selectively or concomitantly induce thrombocyte and monocyte toxicity may limit the efficacy of bevacizumab combination therapy, although neutropenia seems of minor importance in this context. (Of note, lymphocyte toxicity has not been investigated by us.)

Because the clinical assessment of treatment-related toxicities generally do not report on monocyte counts, we used the incidence of grades 3 and 4 thrombocytopenia as a surrogate marker to identify myelosuppressive chemotherapies unlikely to synergize with bevacizumab
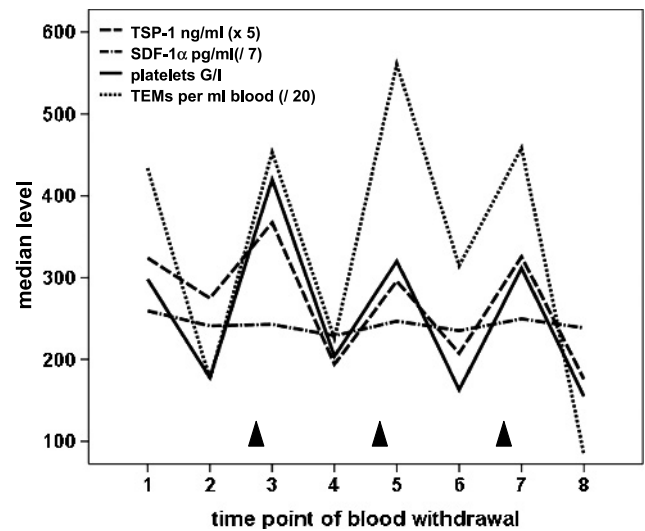

Figure 5. Concurrent parameter fluctuations in accordance with chemotherapy schedule. Median values of platelets, TSP-1 (multiplied by 5), SDF-1α (divided by 7), and TEMs (divided by 20) as established throughout neoadjuvant cancer therapy are depicted in relation to gemcitabine administration. Therapy breaks (preceding blood withdrawal) are indicated by arrowheads.

therapy. It should be noted that we specifically focused on phase 3 trials that directly compared the impact of chemotherapeutic agents within the same study [32–35]. Strikingly, the regimens that had been identified by Shaked et al. to induce proangiogenic cell recruitment (e.g., taxanes and 5-FU) were less commonly associated with platelet reduction compared with the "nonmobilizing" agents gemcitabine and doxorubicin (Table 1). Moreover, the comparison of two studies with advanced NSCLC patients receiving bevacizumab in combination with distinct chemotherapies revealed remarkable associations between efficacy and thrombocytopenia [4,36]. Patients were treated with platin-based chemotherapy plus paclitaxel or gemcitabine in the absence or presence of bevacizumab (at 15 mg/kg). The combination of "mobilizing" paclitaxel and bevacizumab increased median PFS by 1.7 months compared with chemotherapy without VEGF blockade. In contrast, the combination of "nonmobilizing" gemcitabine plus bevacizumab prolonged PFS only marginally by 0.4 months. Furthermore, OS was only prolonged when bevacizumab was applied in combination with paclitaxel [4,37]. Of note, grades 3 and 4 thrombocytopenia were recorded in 1.6% of paclitaxel-treated patients as opposed to 23% of patients receiving gemcitabine.

Because platelets are known to contribute to angiogenesis by pathways distinct from SDF-1α release and EPC mobilization [23], it was of interest to evaluate if thrombocytopenia would translate into

Table 1. Incidence of Grades 3 and 4 Thrombocytopenia Associated with Distinct Chemotherapies.

	Burris et al. [32]		Chan et al. [33]		Joensuu et al. [34]		Albain et al. [35]	
Disease	Advanced pancreatic cancer		Metastatic breast cancer		Advanced breast cancer		Metastatic breast cancer	
No. patients	126		326		237		529	
Chemotherapy	5-FU	Gemcitabine	Docetaxel	Doxorubicin	Docetaxel	Docetaxel alternating gemcitabine	Paclitaxel	Paclitaxel + gemcitabine
Incidence of thrombocytopenia ≥ grade 3	1.6%	9.7%	1.3%*	7.5%*	0%	4.1%	0%	6.1%

*Only grade 4 events were reported.

fluctuations of angiogenic factors that are comprised in platelets. In this context, proangiogenic bFGF and PD-ECGF were found to be largely unaffected by the "drop-and-rebound" changes in platelet counts. Also, VEGF induction by bevacizumab seemed to be independent of thrombocytopenia. This suggests that the circulating proangiogenic factors investigated (VEGF, bFGF, PD-ECGF) were not predominantly platelet-derived but may have been released by tumor or host cell sources other than platelets. In contrast, a highly significant correlation with platelet counts was observed for the antiangiogenic protein TSP-1 (Figure 5), which is in line with our previous investigation suggesting platelets as the major determinant for circulating TSP-1 [38]. The potent angiogenesis inhibitor TSP-1 is increasingly recognized to be a critical mediator of anticancer effects. Expression and release of TSP-1 by platelets direct the host response to suppress tumor growth through inhibiting tumor angiogenesis [24,39]. Furthermore, TSP-1 has been shown to mediate the antiangiogenic effects of metronomic chemotherapy [40]. On the basis of this knowledge, we propose that the repetitive reduction of TSP-1 by thrombocytopenia represents a proangiogenic side effect of gemcitabine therapy not influenced by bevacizumab, which may further limit the benefits of VEGF blockade in combination with gemcitabine.

In conclusion, chemotherapeutic regimens that repeatedly presented unfavorable in combination with bevacizumab share the common feature of thrombocytopenia. Although there may be additional obstacles to antiangiogenic treatment, myelosuppression of thrombocytes and monocytes seems to effectively prevent the mobilization of proangiogenic cell populations (EPCs and TEMs), which are the target of anti-VEGF therapy and the point of synergy in successful combinatorial regimens. Furthermore, the pronounced thrombocytopenia results in a selective loss of the angiogenesis inhibitor TSP-1, which may further explain the disappointing results of myelosuppressive chemotherapeutics in combination with antiangiogenic treatment. Thus, determining the myelosuppressive effect of chemotherapy on thrombocyte and monocyte counts during the active phase of treatment may help to select or exclude agents for combination therapy. Future clinical trials will have to elucidate if bevacizumab combined with chemotherapies of low myelosuppressive activity is able to improve patient outcome for tumor types like pancreatic cancer, which have previously not profited from anti-VEGF therapy.

Acknowledgments

References

[1] Folkman J (1971). Tumor angiogenesis: therapeutic implications. *N Engl J Med* **285**, 1182–1186.
[2] Kilarski WW and Bikfalvi A (2007). Recent developments in tumor angiogenesis. *Curr Pharm Biotechnol* **8**, 3–9.
[3] Verheul HM and Pinedo HM (2003). Vascular endothelial growth factor and its inhibitors. *Drugs Today (Barc)* **39**(suppl C), 81–93.
[4] Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R, and Johnson DH (2006). Paclitaxel-carboplatin alone or with bevacizumab for non–small-cell lung cancer. *N Engl J Med* **355**, 2542–2550.
[5] Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, et al. (2004). Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* **350**, 2335–2342.
[6] Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez EA, Shenkier T, Cella D, and Davidson NE (2007). Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N Engl J Med* **357**, 2666–2676.

[7] Gasparini G, Longo R, Fanelli M, and Teicher BA (2005). Combination of antiangiogenic therapy with other anticancer therapies: results, challenges, and open questions. *J Clin Oncol* **23**, 1295–1311.
[8] Van Cutsem E, Vervenne WL, Bennouna J, Humblet Y, Gill S, Van Laethem JL, Verslype C, Scheithauer W, Shang A, Cosaert J, et al. (2009). Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *J Clin Oncol* **27**, 2231–2237.
[9] Kindler HL, Niedzwiecki D, Hollis D, Sutherland S, Schrag D, Hurwitz H, Innocenti F, Mulcahy MF, O'Reilly E, Wozniak TF, et al. (2010). Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the cancer and leukemia group B (CALGB 80303). *J Clin Oncol* **28**, 3617–3622.
[10] Baeriswyl V and Christofori G (2009). The angiogenic switch in carcinogenesis. *Semin Cancer Biol* **19**, 329–337.
[11] Karayiannakis AJ, Bolanaki H, Syrigos KN, Asimakopoulos B, Polychronidis A, Anagnostoulis S, and Simopoulos C (2003). Serum vascular endothelial growth factor levels in pancreatic cancer patients correlate with advanced and metastatic disease and poor prognosis. *Cancer Lett* **194**, 119–124.
[12] Seo Y, Baba H, Fukuda T, Takashima M, and Sugimachi K (2000). High expression of vascular endothelial growth factor is associated with liver metastasis and a poor prognosis for patients with ductal pancreatic adenocarcinoma. *Cancer* **88**, 2239–2245.
[13] Prewett M, Huber J, Li Y, Santiago A, O'Connor W, King K, Overholser J, Hooper A, Pytowski B, Witte L, et al. (1999). Antivascular endothelial growth factor receptor (fetal liver kinase 1) monoclonal antibody inhibits tumor angiogenesis and growth of several mouse and human tumors. *Cancer Res* **59**, 5209–5218.
[14] Solorzano CC, Baker CH, Bruns CJ, Killion JJ, Ellis LM, Wood J, and Fidler IJ (2001). Inhibition of growth and metastasis of human pancreatic cancer growing in nude mice by PTK 787/ZK222584, an inhibitor of the vascular endothelial growth factor receptor tyrosine kinases. *Cancer Biother Radiopharm* **16**, 359–370.
[15] Shaked Y, Henke E, Roodhart JM, Mancuso P, Langenberg MH, Colleoni M, Daenen LG, Man S, Xu P, Emmenegger U, et al. (2008). Rapid chemotherapy-induced acute endothelial progenitor cell mobilization: implications for antiangiogenic drugs as chemosensitizing agents. *Cancer Cell* **14**, 263–273.
[16] Jin DK, Shido K, Kopp HG, Petit I, Shmelkov SV, Young LM, Hooper AT, Amano H, Avecilla ST, Heissig B, et al. (2006). Cytokine-mediated deployment of SDF-1 induces revascularization through recruitment of CXCR4$^+$ hemangiocytes. *Nat Med* **12**, 557–567.
[17] Avecilla ST, Hattori K, Heissig B, Tejada R, Liao F, Shido K, Jin DK, Dias S, Zhang F, Hartman TE, et al. (2004). Chemokine-mediated interaction of hematopoietic progenitors with the bone marrow vascular niche is required for thrombopoiesis. *Nat Med* **10**, 64–71.
[18] Roodhart JM, Langenberg MH, Vermaat JS, Lolkema MP, Baars A, Giles RH, Witteveen EO, and Voest EE (2010). Late release of circulating endothelial cells and endothelial progenitor cells after chemotherapy predicts response and survival in cancer patients. *Neoplasia* **12**, 87–94.
[19] Brostjan C, Bayer A, Zommer A, Gornikiewicz A, Roka S, Benko T, Yaghubian R, Jakesz R, Steger G, Gnant M, et al. (2003). Monitoring of circulating angiogenic factors in dendritic cell–based cancer immunotherapy. *Cancer* **98**, 2291–2301.
[20] Brostjan C, Gebhardt K, Gruenberger B, Steinrueck V, Zommer H, Freudenthaler H, Roka S, and Gruenberger T (2008). Neoadjuvant treatment of colorectal cancer with bevacizumab: the perioperative angiogenic balance is sensitive to systemic thrombospondin-1 levels. *Clin Cancer Res* **14**, 2065–2074.
[21] Willett CG, Boucher Y, Duda DG, di Tomaso E, Munn LL, Tong RT, Kozin SV, Petit L, Jain RK, Chung DC, et al. (2005). Surrogate markers for antiangiogenic therapy and dose-limiting toxicities for bevacizumab with radiation and chemotherapy: continued experience of a phase I trial in rectal cancer patients. *J Clin Oncol* **23**, 8136–8139.
[22] 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.
[23] Klement GL, Yip TT, Cassiola F, Kikuchi L, Cervi D, Podust V, Italiano JE, Wheatley E, Abou-Slaybi A, Bender E, et al. (2009). Platelets actively sequester angiogenesis regulators. *Blood* **113**, 2835–2842.
[24] Zaslavsky A, Baek KH, Lynch RC, Short S, Grillo J, Folkman J, Italiano JE Jr, and Ryeom S (2010). Platelet-derived thrombospondin-1 is a critical negative regulator and potential biomarker of angiogenesis. *Blood* **115**, 4605–4613.
[25] Eskens FA and Sleijfer S (2008). The use of bevacizumab in colorectal, lung, breast, renal and ovarian cancer: where does it fit? *Eur J Cancer* **44**, 2350–2356.

[26] Brufsky A, Rivera RR, Hurvitz SA, Bondarenko IN, Smirnov V, Valero V, Rugo HS, Swamy R, Mu H, and Perez EA (2010). Progression-free survival (PFS) in patient subgroups in RIBBON-2, a phase III trial of chemotherapy (chemo) plus or minus bevacizumab (BV) for second-line treatment of HER2-negative, locally recurrent or metastatic breast cancer (MBC). *J Clin Oncol (ASCO Annual Meeting Proceedings)* **28**. Abstract 1021.

[27] Markman M (2003). Managing taxane toxicities. *Support Care Cancer* **11**, 144–147.

[28] Brignone C, Gutierrez M, Mefti F, Brain E, Jarcau R, Cvitkovic F, Bousetta N, Medioni J, Gligorov J, Grygar C, et al. (2010). First-line chemoimmunotherapy in metastatic breast carcinoma: combination of paclitaxel and IMP321 (LAG-3Ig) enhances immune responses and antitumor activity. *J Transl Med* **8**, 71.

[29] Tong AW, Seamour B, Lawson JM, Ordonez G, Vukelja S, Hyman W, Richards D, Stein L, Maples PB, and Nemunaitis J (2000). Cellular immune profile of patients with advanced cancer before and after taxane treatment. *Am J Clin Oncol* **23**, 463–472.

[30] Kitayama J, Yasuda K, Kawai K, Sunami E, and Nagawa H (2010). Circulating lymphocyte number has a positive association with tumor response in neoadjuvant chemoradiotherapy for advanced rectal cancer. *Radiat Oncol* **5**, 47.

[31] Saif MW, Choma A, Salamone SJ, and Chu E (2009). Pharmacokinetically guided dose adjustment of 5-fluorouracil: a rational approach to improving therapeutic outcomes. *J Natl Cancer Inst* **101**, 1543–1552.

[32] Burris HA III, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, et al. (1997). Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* **15**, 2403–2413.

[33] Chan S, Friedrichs K, Noel D, Pinter T, Van Belle S, Vorobiof D, Duarte R, Gil Gil M, Bodrogi I, Murray E, et al. (1999). Prospective randomized trial of docetaxel versus doxorubicin in patients with metastatic breast cancer. *J Clin Oncol* **17**, 2341–2354.

[34] Joensuu H, Sailas L, Alanko T, Sunela K, Huuhtanen R, Utriainen M, Kokko R, Bono P, Wigren T, Pyrhonen S, et al. (2010). Docetaxel versus docetaxel alternating with gemcitabine as treatments of advanced breast cancer: final analysis of a randomised trial. *Ann Oncol* **21**, 968–973.

[35] Albain KS, Nag SM, Calderillo-Ruiz G, Jordaan JP, Llombart AC, Pluzanska A, Rolski J, Melemed AS, Reyes-Vidal JM, Sekhon JS, et al. (2008). Gemcitabine plus paclitaxel versus paclitaxel monotherapy in patients with metastatic breast cancer and prior anthracycline treatment. *J Clin Oncol* **26**, 3950–3957.

[36] Reck M, von Pawel J, Zatloukal P, Ramlau R, Gorbounova V, Hirsh V, Leighl N, Mezger J, Archer V, Moore N, et al. (2009). Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for non-squamous non–small-cell lung cancer: AVAil. *J Clin Oncol* **27**, 1227–1234.

[37] Reck M, von Pawel J, Zatloukal P, Ramlau R, Gorbounova V, Hirsh V, Leighl N, Mezger J, Archer V, Moore N, et al. (2010). Overall survival with cisplatin-gemcitabine and bevacizumab or placebo as first-line therapy for nonsquamous non–small-cell lung cancer: results from a randomised phase III trial (AVAiL). *Ann Oncol* **21**, 1804–1809.

[38] Starlinger P, Moll HP, Assinger A, Nemeth C, Hoetzenecker K, Gruenberger B, Gruenberger T, Kuehrer I, Schoppmann SF, Gnant M, et al. (2010). Thrombospondin-1: a unique marker to identify *in vitro* platelet activation when monitoring *in vivo* processes. *J Thromb Haemost* **8**, 1809–1819.

[39] Kazerounian S, Yee KO, and Lawler J (2008). Thrombospondins in cancer. *Cell Mol Life Sci* **65**, 700–712.

[40] Bocci G, Francia G, Man S, Lawler J, and Kerbel RS (2003). Thrombospondin 1, a mediator of the antiangiogenic effects of low-dose metronomic chemotherapy. *Proc Natl Acad Sci USA* **100**, 12917–12922.