Tissue factor expressed by circulating cancer cell-derived microparticles drastically increases the incidence of deep vein thrombosis in mice

G. M. THOMAS,*†‡ A. BRILL,*†§ S. MEZOUAR,‡ L. CRESCENCE,‡ M. GALLANT,* C. DUBOIS‡ and D. D. WAGNER*†¶

*Program in Cellular and Molecular Medicine, Boston Children’s Hospital; †Department of Pediatrics, Harvard Medical School, Boston, MA, USA; ‡VRCM, Aix Marseille Université, Inserm UMR-S 1076, Marseille, France; §Centre for Cardiovascular Sciences, Institute of Biomedical Research, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK; and ¶Division of Hematology/Oncology, Boston Children’s Hospital, Boston, MA, USA


Summary. Background: The risk of thrombotic complications such as deep vein thrombosis (DVT) during tumor development is well known. Tumors release into the circulation procoagulant microparticles (MPs) that can participate in thrombus formation following vessel injury. The importance of this MP tissue factor (TF) in the initiation of cancer-associated DVT remains uncertain. Objective: To investigate how pancreatic cancer MPs promote DVT in vivo. Methods: We combined a DVT mouse model in which thrombosis is induced by flow restriction in the inferior vena cava with one of subcutaneous pancreatic cancer in C57BL/6J mice. We infused high-TF and low-TF tumor MPs to determine the importance of TF in experimental cancer-associated DVT. Results: Both tumor-bearing mice and mice infused with tumor MPs subjected to 3 h of partial flow restriction developed an occlusive thrombus; fewer than one-third of the control mice did. We observed that MPs adhered to neutrophil extracellular traps (NETs), which are functionally important players during DVT, whereas neither P-selectin nor glycoprotein Ib were required for MP recruitment in DVT. The thrombotic phenotype induced by MP infusion was suppressed by hirudin, suggesting the importance of thrombin generation. TF carried by tumor MPs was essential to promote DVT, as mice infused with low-TF tumor MPs had less thrombosis than mice infused with high-TF tumor MPs. Conclusions: TF expressed on tumor MPs contributes to the increased incidence of cancer-associated venous thrombosis in mice in vivo. These MPs may adhere to NETs formed at the site of thrombosis.

Keywords: cancer; cell-derived microparticles; neutrophils; tissue factor; venous thrombosis.

Introduction
The prevalence of deep vein thrombosis (DVT) in cancer patients is up to six-fold higher than in the general population [1–3]. Cancer patients are also more susceptible to recurrent venous thromboembolism (VTE) [4–8], and this risk is particularly high in patients with pancreatic cancer [9–11]. VTE events contribute greatly to morbidity and mortality, and also challenge physicians by delaying, interrupting or interfering with cancer therapy [12].

Tumor cells induce and take advantage of the hypercoagulable state, which promotes tumor growth [13], angiogenesis [14], and metastasis [15–17]. The ability of cancer cells to induce platelet aggregation was first observed by Gasic et al. more than 30 years ago [18], and has been the focus of several studies [19–21]. However, the factors that promote cancer-associated DVT require further study.

In a model of ferric chloride-induced injury, it was previously demonstrated that tumor-bearing mice have shortened occlusion times in mesenteric venules and arterioles as compared with control mice [22]. This work indicated that the thrombotic phenotype was attributable to the shedding of procoagulant microparticles (MPs) from cancer cells into blood. These MPs express a higher density of active tissue factor (TF) than the parental cell (> 100-fold), conferring an ability to promote vessel injury-
induced thrombosis [22,23]. The presence of tumor MPs has been observed in the circulation of both mice and humans, and several studies have suggested a positive correlation between circulating TF-bearing MPs and the occurrence of thrombotic events in cancer patients. The available data are ambiguous [11,24–26]. Thaler et al. have shown that MP TF activity was not associated with future VTE but rather with ongoing or past VTE and mortality. However, no serial measurements were performed, with only one blood sample being taken on the day of study initiation. It is still possible that MP TF activity increased at a later stage of the disease or before patients developed VTE. In the present study, we investigated whether tumors and/or tumor MPs could promote DVT initiation in mice. We used an established murine model of DVT by producing stenosis in the inferior vena cava (IVC). The model recapitulates the blood flow conditions in human deep vein valves: turbulence, whirling, lack of laminarity, and local hypoxia. With these conditions as a common denominator, we superposed cancer cells or MPs as a variable. The mouse model approximates as cannot mimic DVT pathogenesis in cancer patients; yet, it addresses the effect of cancer on the venous thrombosis process. In contrast to most DVT models, our model maintains blood flow in the IVC, and does not induce endothelial injury or denudation [27]. Also, the IVC side branches are ligated so that they do not influence thrombus development [28]. The pathologic venous thrombi form by a complex process involving endothelial release of von Willebrand factor (VWF) [27], and recruitment and cooperation of platelets, neutrophils, monocytes, and red blood cells [27,29–31]. In the absence of cancer, studies have shown that neutrophils play an essential role in this thrombotic process by forming neutrophil extracellular traps (NETs) [29,30,32].

The mechanisms behind the increased risk of DVT during cancer development and whether circulating tumor MPs can trigger DVT in stenotic vessels are not known. Wang et al. previously reported that TF of tumor origin can affect venous thrombosis in a ferric chloride model of saphenous vein injury in nude mice [33]. With a C57BL/6J syngeneic cancer model, DVT was tested in wild-type C57BL/6J mice with or without murine pancreatic cancer [22], giving us the advantage of using immunocompetent mice. In addition, we were able to show that infusion of tumor MPs promotes DVT even in healthy mice.

Materials and methods

Mice

Experiments were performed with wild-type (WT) C57BL/6J mice purchased from the Jackson Laboratory (Bar Harbor, ME, USA) or P-selectin−/− mice [34] of the same genetic background. All mice were 3–8-week-old males housed in the animal facility at Boston Children’s Hospital. Tumor cells (or control phosphate-buffered saline [PBS]) were injected subcutaneously into 3-week-old mice, and DVT was then induced at 8 weeks of age in the same mice. Experimental procedures were approved by the Animal Care and Use Committee of Boston Children’s Hospital.

Cell lines

The Panc02 cell line was derived from a pancreatic ductal adenocarcinoma induced in a C57BL/6 mouse [35]. Panc02 cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U mL−1 penicillin, 100 μg mL−1 streptomycin, and 0.1% fungizone. Blasticidin was used as the selection antibiotic for the Panc02 low-TF cell line described below. Cells were grown at 37 °C in a humidified atmosphere with 5% CO2.

The Panc02-LowTF clone has been described previously [23]. The Panc02 cells were stably transfected with the TF-silencing vector by the use of Lipofectamine 2000 and PLUS reagent (Life Technologies, Carlsbad, CA, USA). Cells were sorted for green fluorescent protein expression (which was a function of the TF knockdown level) with a FACS Aria III (BD Biosciences, Franklin Lakes, NJ, USA). TF expression was further assessed by RT-PCR, immunofluorescence and the TF activity measurement described in a previously published study [23].

MP preparation

The isolation of MPs was performed as previously described [22], and their concentration was determined by protein quantification with the bicinchoninic acid assay method (Thermo Fisher Scientific, Waltham, MA, USA). The concentration of MPs used in vivo (0.2 μg g−1 mouse body weight) has been determined by flow cytometry analysis, and shown to correspond to 18 000 MPs g−1 mouse body weight [22].

Induction of ectopic tumors

Induction of ectopic tumors with Panc02 cells was performed as previously described [22], by subcutaneous injection of 2 × 106 cells into the right flank of 3-week-old mice, and the tumors were grown for 5 weeks.

IVC stenosis model

The flow restriction model was as previously described [27,29]. Briefly, mice were anesthetized with isoflurane–oxygen mixture, and, after laparotomy, the IVC was separated from the aorta and ligated below the left renal vein over a spacer (0.26 mm in diameter) that was removed immediately after ligation. All visible side branches were ligated. This procedure decreases the vascular lumen by
~ 90% (in 8-week-old mice), and produces no detectable endothelial denudation. Mice were killed 1 h, 3 h or 48 h after stenosis induction, and thrombi developed below the suture (in the caudal direction). In this DVT model, IVC ligation followed by quick removal of the suture does not induce thrombus development in 48 h or endothelial activation in 6 h [27], indicating that thrombosis is induced by flow restriction and not by the ligation procedure. Some mice were given intravenous Panc02-derived MPs (0.2 μg of MP-associated proteins per gram of body weight) or PBS immediately after stenosis induction.

GPG290 treatment was performed as previously described [27]. Values for the weight and length of each thrombus were deemed to be 0 when no thrombus was detected in the IVC.

**Intravital microscopy and quantification of accumulated MPs**

Mice were anesthetized with isoflurane-oxygen mixture and kept under anesthesia for the whole experiment. To visualize MP accumulation during thrombus formation in the IVC, calcein-AM-labeled cancer cell-derived MPs (0.2 μg g−1 of body weight) were injected intravenously, just after induction of IVC stenosis, via a catheter implanted into the left jugular vein. Accumulation of fluorescent MPs in the IVC was assessed by intravital fluorescence microscopy 10 min, 30 min and 60 min after induction of stenosis and MP injection. The fluorescent background was recorded before MP injection, and was digitally removed from each acquisition following injection. Fluorescent images were acquired with a Zeiss Axioplan upright fluorescence microscope with an LED 4-Color Light Engine (Lumencor, Beaverton, OR, USA), in conjunction with a VGA CCD Camera (Hamamatsu, Bridgewater, NJ, USA) and an image intensifier (VideoScope, Dulles, VA, USA), SLIDEBOOK 5.0 software (Intelligent Imaging Innovations, Denver, CO, USA) was used for control of all hardware components, digital acquisition, and data analysis.

**Assay of adhesion of MPs to NETs**

Neutrophils were isolated on a Percoll gradient from WT mouse bone marrow. Neutrophils were then stimulated with 50 μM platelet-activating factor-16 (Merck Millipore, Molsheim, France), a potent inducer of NETosis released by many cells involved in host defense [36]. The cells were washed with PBS, incubated with DiD-labeled Panc02-derived MPs (2 μg mL−1 final concentration) for 10 min at 37 °C, and washed again. The preparation was then fixed and stained with Hoechst 33342 before visualization on a motorized Leica DMi8000 B fluorescence microscope (Buffalo Grove, IL, USA) in conjunction with a CCD Hamamatsu Orea ER2 camera. For each independent experiment, three wells were analyzed per condition, and at least three random photographs were taken per well. Representative pictures were then chosen for each condition. Flow experiments were performed in flow chambers (μ-Slide IV; IBIDI, Marne la Vallée, France) at a flow rate of 100 s−1. If indicated, DNase-I (50 U mL−1; Pulmozyme; Roche, Boulogne-Billancourt, France) was added before MP perfusion. MP binding was visualized with a motorized Leica DMI8 fluorescence microscope in conjunction with a Leica DFC3000G camera. METAMORPH software (Molecular Devices, Sunnyvale, CA, USA) was used for digital acquisition.

**Statistical analysis**

We assessed statistical significance for non-parametric distributions with the two-tailed Mann–Whitney test. The proportions of mice that developed a thrombus were compared between the different experimental groups by use of the Fisher test. Differences were considered to be significant at \( P < 0.05 \).

**Results**

**Tumor or tumor-derived MPs promote DVT**

To analyze whether cancer cell-derived MPs promote DVT initiation in vivo, we used an established murine model of Trousseau’s syndrome. In this model, a subcutaneous injection of pancreatic cancer cells leads to the development of an ectopic tumor and the presence of circulating tumor-derived MPs in the bloodstream [22]. To determine whether the model was suitable for studying cancer-associated DVT, we combined it with a model of DVT induced by IVC stenosis. Using the two models together, we observed that all of the tumor-bearing mice (eight of eight) developed a thrombus within 3 h after flow restriction of the IVC, whereas fewer than one-third of mice without tumor (two of seven) developed a thrombus (Fig. 1). We have previously shown that these tumors shed TF-bearing MPs, which alone can accelerate thrombus formation following vessel injury [22]. Interestingly, when Panc02 cell-derived MPs (0.2 μg g−1 mouse body weight) were infused into the bloodstream of healthy mice 5 min after IVC stenosis, the occurrence of thrombus formation (10 of 10) was identical to that in tumor-bearing mice (eight of eight) (Fig. 1A), and the medians of thrombus weights and sizes were similar to those in the tumor-bearing mouse group (Fig. 1B-C). To determine whether cancer cell-derived MPs accumulate at the IVC stenosis site, MPs were isolated, labeled with the calcein-AM fluorochrome, and infused into a recipient mouse after stenosis induction. Using intravital microscopy, within minutes we detected the presence of fluorescently labeled MPs accumulating at the site of the growing thrombus (Fig. 1D). These results suggest that blood flow restriction induces tumor MP accumulation at the
site of thrombosis, and that this could promote the formation of cancer-associated DVT in vivo.

**P-selectin and glycoprotein (GP)Ib are not required for MP promotion of DVT while MPs bind to NETs**

The TF-bearing tumor MPs used in this study have been shown to target sites of vessel injury through interaction of P-selectin GP ligand-1 (PSGL-1) expressed at their surface with P-selectin expressed at a site of vessel injury [22]. Blood flow restriction induces endothelial activation below the stenosis site, leading to local degranulation of Weibel–Palade bodies (WPBs) from endothelial cells [27]. Because P-selectin and VWF are stored in WPBs, we first tested, in a blinded experiment, whether cancer cell-derived MPs were still able to promote DVT when the P-selectin–PSGL-1 axis was disrupted. We infused cancer cell-derived MPs into the circulation of P-selectin–/– mice, and induced stenosis of the IVC. After 3 h of blood flow restriction, all of the P-selectin–/– mice infused with MPs (n = 7) developed DVT, whereas only one-third of the P-selectin–/– mouse group infused with PBS did so (two of six) (Fig. 2A). Thrombi from MP-infused P-selectin–/– mice had medians of thrombus weight (Fig. 2B) and length (Fig. 2C) comparable to those of thrombi observed in MP-infused WT mice. These data suggest that cancer cell-derived MPs do not require P-selectin for their recruitment to promote DVT in this model.

Next, we examined whether the MPs could be recruited by platelet GPIb, which binds to Mac-1, P-selectin, and other ligands such as VWF [37–39]. Concomitantly with the MPs, we infused GPG290, a mutant GPIb-immunoglobulin chimera that inhibits interaction of the VWF A1 domain and probably competes with other GPIb-mediated interactions. This chimera has been shown to prevent thrombosis in WT healthy mice after 48 h of stenosis in this DVT model [27]. However, we observed that, when mice were infused with tumor MPs and GPG290, thrombosis was still observed as early as 1 h after stenosis (Fig. 2D–F), suggesting that MPs do not require GPIb or the VWF A1 domain to promote DVT, and can induce DVT even if VWF-mediated platelet adhesion is prevented. As an internal control, we verified that, in this study, GPG290 also inhibited thrombus formation in healthy WT mice after 48 h of IVC stenosis (Fig. S1).

It was recently shown that NETs constitute an important part of deep vein thrombi [29,30] and that NETs form early in the DVT process [32]. Interestingly, we observed that cancer cell-derived MPs can adhere to NETs in vitro both under static conditions (Fig. 2G) and flow conditions (Fig. 2H). Treatment with DNase-1 prevented MP adhesion, further indicating that the MPs bound to NETs (Fig. 2H). These results suggest that NETs could recruit MPs to the site of the pathologic thrombosis.

**TF carried by cancer cell-derived MPs is essential for their DVT-promoting activity**

In the mouse model of DVT used in this study, thrombus formation is associated with endothelium activation without any exposure of the subendothelial matrix. TF-dependent thrombin generation has been shown to be...
Fig. 2. P-selectin and GPIb are not required for MP promotion of DVT while MPs bind to NETs. (A–C) Three-hour inferior vena cava (IVC) stenosis in control P-selectin-deficient mice (PselKO + PBS, n = 6) and in P-selectin-deficient mice infused with cancer cell-derived microparticles (MPs) (PselKO + MPs, n = 7). (A) Percentage of mice with a thrombus. (B, C) Values for weight and length of each thrombus. The horizontal bars in dot plots represent medians. (D–F) One-hour IVC stenosis in GPG290-pretreated mice (n = 9) and in GPG290-pretreated mice infused with cancer cell-derived MPs (n = 7). (D) Percentage of mice with a thrombus. (E, F) Values for weight and length of each thrombus. (G, H) Representative images showing Panc02-derived MPs interacting with neutrophil extracellular traps (NETs) but not with unstimulated neutrophils. MPs were labeled with DiD (red), and NET DNA was stained with Hoechst 33342 (blue) after incubation of MPs with washed mouse neutrophils preincubated in the absence (no stimulation) or in the presence of 50 µM platelet-activating factor (PAF) to induce NET formation. (G) Experiment performed under static conditions. The photograph on the right is at higher magnification, as indicated by the bar. (H) Experiment performed in a flow chamber, at a shear rate of 100 s⁻¹. The flow direction was from left to right. The photograph on the right shows the absence of MP binding after treatment with DNase-1. PBS, phosphate-buffered saline.
of importance in DVT [32,40,41]. Because cancer cell-derived MPs are very rich in active TF, we examined whether they were able to trigger DVT independently of TF expressed by long-term-activated endothelial cells. We therefore performed stenosis for a short period, i.e. 1 h, a time that is probably too brief for induction of synthesis and expression of TF [42]. We observed that nearly two-thirds of the mice infused with cancer cell-derived MPs (eight of 13) still developed a thrombus after 1 h of stenosis, whereas only one of nine of the control mice did so (Fig. 3). Not surprisingly, we observed that cancer cell-derived MP-induced DVT was completely abolished when thrombin generation was inhibited by hirudin infusion (Fig. 3A). In order to further establish the importance of TF expressed on cancer cell-derived MPs, we isolated MPs from low-TF Panc02 cells [23]. Interestingly, the perfusion of low-TF MPs significantly reduced the incidence of thrombus formation in this model, and also significantly reduced thrombus weight and size as compared with mice perfused with unmodified Panc02-derived MPs (Fig. 3D–F). Altogether, these observations show that, in our model, cancer cell-derived MPs express enough TF at their surface to significantly increase the incidence of DVT in vivo.

Discussion
In order to better understand how cancer promotes DVT, we used a mouse model of DVT induced by IVC stenosis in combination with a mouse model of pancreatic cancer. In contrast to most DVT models [43–45], our model maintains blood flow and does not induce injury [27]. Here, we showed that all tumor-bearing mice already formed an occlusive thrombus after 3 h of stenosis, whereas the incidence of thrombus formation was low in mice without cancer. Previous studies have shown that these pancreatic tumors release into the circulation MPs that accelerate thrombus formation following injury [29,30]. The MPs express PSGL-1, allowing them to bind P-selectin presented by activated endothelium or platelets, and active TF, which can trigger blood coagulation [22]. We have now observed that, when MPs are infused into healthy mice, they also trigger DVT. However, in the model of blood flow restriction, the MPs did not require P-selectin for their recruitment and activity, because P-selectin-deficient mice were as susceptible to MP-induced DVT as WT mice. MP recruitment could possibly be supported by integrins, as Panc02-derived MPs have recently been shown to express CD29 (αν), CD51 (β1), and CD61 (β3), whose inhibition by RGDV peptide prevented their binding to fibrinogen under flow [23]. We previously observed that free circulating Panc02 MPs have a half-life of ~10 min in mice [22]. However, these MPs expressing PSGL-1 [22] and integrins [23] at their surface could stick to activated endothelium, platelets, leukocytes, or fibrin. This might explain the increased accumulation of fluorescent tumor MPs in the thrombus over time. In addition, we observed that the cancer MPs bound avidly to NETs, which are known to be formed early in this DVT model and to play a crucial role in the thrombosis process.

Fig. 3. Tissue factor (TF) carried by tumoral MPs is essential for their DVT-promoting activity. (A–C) One-hour inferior vena cava (IVC) stenosis in control wild-type (WT) mice (n = 9), in mice infused intravenously with cancer cell-derived microparticles (MPs) (n = 13), and in mice infused with cancer cell-derived MPs and pretreated with hirudin (MPs + Hir, hirudin 8 U g−1, n = 6). (A) Percentage of mice with a thrombus. (B, C) Values for weight and length of each thrombus. The horizontal bars in dot plots represent medians. (D–F) One-hour IVC stenosis in WT mice infused with unmodified Panc02-derived MPs (n = 19) and in mice infused with low-TF MPs (n = 19). (D) Percentage of mice with a thrombus. (E, F) Values for weight and length of each thrombus. The horizontal bars in dot plots represent medians.
The observation of tumor MPs interacting with NETs is consistent with a study showing that histones, which form part of NETs, bind phospholipids such as phosphatidylserine and phosphatidylethanolamine present on MPs [46]. Our results may explain a recent finding in a cancer patient by Thaler et al. of TF-positive cerebral and myocardial thrombi containing both epithelial markers (probably from tumor MPs) and citrullinated histone H3, which is indicative of the presence of NETs [47].

The present study complements well that of Wang et al., who observed that human tumors expressing TF implanted in nude mice activated coagulation in these mice and increased thrombosis in a ferric chloride model of saphenous vein injury [33]. However, in their limited study of DVT without ligation of side branches [28], they did not detect differences in thrombus weight. Our larger study using syngeneic murine tumors or tumor MPs shows the direct involvement of TF expressed on cancer cell-derived MPs in DVT incidence. We chose not to evaluate thrombosis in mice bearing low-TF Panc02 tumors, because tumor growth is much reduced in these mice [23], which would complicate data interpretation. These mouse studies may help to explain the observed correlation between circulating MP TF activity and the heightened occurrence of VTE events in certain types of cancer patient [11]. It is possible that patients develop VTE following a rise in plasmatic MP TF activity, which could trigger thrombosis. Interestingly, the surface expression of TF on cancer cells may increase with the malignancy grade of the tumor [48], making it a marker of poor prognosis.

The importance of MP-linked TF in the triggering of venous thrombosis is further supported by the fact that phospholipids present on MPs, such as phosphatidylethanolamine and phosphatidylserine, strongly increase their procoagulant activity [49], and we have shown that their procoagulant activity may replace that of platelets when platelet recruitment is decreased by GPG290 (Fig. 2D–F; Fig. S1). However, the observation that thrombus formation still occurs in MP-injected mice despite GP Ibα blockade does not necessarily indicate that MPs are driving thrombus formation without a contribution of platelets. TF-positive circulating MPs may rapidly activate the coagulation system after injection, leading to thrombin generation and platelet activation. A recent study by Davila et al. has confirmed this hypothesis. The authors observed that injection of pancreatic cancer-derived MPs into mice rapidly induced thrombin generation, as shown by elevated thrombin–antithrombin complex levels, and platelet activation leading to thrombocytopenia and signs of shock [50].

Other cancers, such as glioblastoma, can induce a procoagulant state even if they express lower levels of TF. Here, other pathways must be employed to heighten the thrombotic risk. The secretion by the tumor of cytokines or growth factors can affect thrombosis through thrombocytosis [51], leukocytosis [52,53], and NETosis [36], a process that is crucial for DVT [54].

Clinical studies have suggested an advantage of long-term low molecular weight heparin over vitamin K antagonists such as warfarin for patients with VTE in the setting of cancer [55]. This could be explained by the fact that, among its other effects, heparin can dismantle NETs’ scaffold [31] and prevent histone-mediated platelet aggregation [56].

Cancer and its treatment affect the three components of Virchow’s triad, in which imbalance can lead to venous thrombosis: release of procoagulant factors into the circulation, alteration of blood flow, and damage to/activation of endothelial cells. In our DVT model, cancer cell-derived MPs do not seem to require selectin interaction to trigger thrombus formation. However, we believe that, like NETs, P-selectin might be important later in the pathologic recruitment of cancer cell-derived MPs in the thrombus and in thrombus stabilization. For instance, P-selectin has been shown to be implicated in a mouse IVC stasis model at later time points and in the absence of cancer [57]. It would not be surprising if tumor MPs had a worse impact on thrombosis in patients with cancer who are treated with chemotherapy, as such drugs can damage the vascular endothelium [58,59] and induce NETosis [60]. NETs induced by chemotherapy [60] may contribute to MP recruitment and thus to thrombosis. Chemotherapy could also affect the risk of venous thrombosis [61,62] by inducing the production of TF-positive MPs following cancer cells’ apoptosis in response to treatment [63,64], just as leukocyte-derived MPs have been found to be involved in venous thrombogenesis in vivo [57]. In addition, several studies have shown that platelet activation and thrombin generation, and extracellular DNA, can further promote tumor progression and spread [65,66].

Altogether, our results support the hypothesis that tumor MPs and their TF help to trigger cancer-associated venous thrombosis. Thus, targeting their recruitment or activity could provide strategies to prevent the morbidity associated with cancer-related thrombotic complications.

Addendum

G. M. Thomas designed and performed research, collected, analyzed and interpreted the data, and wrote the paper. A. Brill designed and performed research, and interpreted the data. S. M. Mezouar contributed to the preparation and characterization of the low-TF Panc02 cells. L. Crescence performed research. M. Gallant provided valuable technical assistance. C. Dubois provided crucial reagents and supervised the study. D. D. Wagner designed and supervised the study, and wrote the paper.

© 2015 International Society on Thrombosis and Haemostasis
Acknowledgements
We are grateful to L. Cowan for help with the preparation of the manuscript. We thank K. Ketman for technical assistance with cell sorting, and G. D. Shaw from Wyeth Research (Cambridge, MA, USA) for the gift of GPG290. We thank F. Dignat-George for her help and scientific support, and the common research service of the URMITE UMR 6236, CNRS-IRD (Marseille, France) and P. Weber for access to and help with the Leica fluorescence microscope. This work was supported by the National Heart, Lung and Blood Institute of the National Institutes of Health, grant RO1 HL102101 (D. D. Wagner).

Disclosure of Conflict of Interests
The authors state that they have no conflict of interest.

Supporting Information
Additional Supporting Information may be found in the online version of this article:

Fig. S1. Inhibition of DVT after 48 h of stenosis in GPG290-treated mice not carrying a tumor.

References


Ishii H, Horie S, Kizaki K, Kazama M. Retinoic acid counteracts both the downregulation of thrombomodulin and the induc-


