Published in final edited form as:

Thromb Res. 2018 April ; 164(Suppl 1): S48–S53. doi:10.1016/j.thromres.2017.12.018.

Mouse models of cancer-associated thrombosis

Yohei Hisada^{1,2} and Nigel Mackman^{1,3}

¹Department of Medicine, Division of Hematology and Oncology, Thrombosis and Hemostasis Program, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

²K. G. Jebsen Thrombosis Research and Expertise Center, University of Tromsø, Tromsø, Norway

³Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Abstract

Cancer patients have an increased risk of venous thromboembolism (VTE) compared with the general population. Mouse models are used to better understand the mechanisms of cancer-associated thrombosis. Several mouse models of cancer-associated thrombosis have been developed that use different mouse strains, tumors, tumor sites and thrombosis model. In this review, we summarize these different models. These models have been used to determine the role of different pathways in cancer-associated thrombosis. For instance, they have revealed roles for tumor-derived tissue factor-positive microvesicles and neutrophil extracellular traps in thrombosis in tumor-bearing mice. A better understanding of the mechanisms of cancer-associated thrombosis may allow the development of new therapies to reduce thrombosis in cancer patients.

Keywords

cancer; mouse model; neutrophil extracellular traps; tissue factor; venous thrombosis

Introduction

Cancer patients have an increased risk of venous thromboembolism (VTE) compared with the general population. Biomarkers studies have identified a number of distinct pro-coagulant pathways activated in cancer patients that may contribute to VTE [1]. Importantly, the incidence of VTE varies in different types of cancer [2–5]. This suggests that there may be cancer site-specific mechanisms of VTE [6]. However, the underlying mechanisms leading to VTE in each type of cancer have not been elucidated.

Mouse models are used to study mechanisms of cancer-associated thrombosis. A number of different models have been developed that vary by mouse strains, cancer-types and

Address for correspondence: Nigel Mackman, PhD, University of North Carolina at Chapel Hill, 111 Mason Farm Road Campus Box 7126, Chapel Hill, North Carolina, 27599, Telephone: (919) 843 3961, Fax: (919) 966 7639, nigel_mackman@med.unc.edu.

Conflict of interest statement

The authors state that they have no conflict of interest.

thrombosis models employed. This makes direct comparison difficult. Here, we review the different models and the studies on the role of various pathways in cancer-associated thrombosis.

Choice of mouse strain and cancer cell

The strains of mice used to study cancer-associated thrombosis are largely dictated by the type of cancer-cells used. Immunocompetent mouse strains, most commonly C57BL/6 and BALB/c, are used to generate allograft models with murine cancer cells (Table 1). It is important that the murine cancer cell line is compatible with the strain of mice. The major strength of immunocompetent models is that the host is able to mount a full immune response to the tumor that may contribute to thrombosis. In addition, one can analyze the role of a given host protein using knockout mice. However, there are a limited number of murine cancer cell lines available. The three most commonly used murine cell lines for studies on cancer-associated thrombosis are 4T1, Lewis lung carcinoma (LLC) and Panc02. The 4T1 cell line is a thioguanine-resistant derivative of the 410.4 line that was isolated from a spontaneous mammary tumor in BALB/c3H mice [7]. The LLC cell line was isolated from a spontaneous carcinoma in the lung of C57BL/6 mice [8]. The Panc02 cell line was derived from a tumor formed in the pancreas of C57BL/6 mice treated with 3-methyl-chloronitrothrene [9]. Genetically engineered mice, such as the *LSL-Kras^{G12D/+};LSL-Trp53^{R172H/+};Pdx-1-Cre* (KPC) model, develop primary tumors within the pancreas that look and behave like human pancreatic cancer [10]. The KPC model was developed using C57BL/6 mice. Three cell lines (KPC 4684, KPC 4112 and KPC 4580P) have been established from KPC mouse tumors that have been engineered to express luciferase under a *Pdx-1-Cre* promoter (Dr. J-J Yeh, unpublished data). We are currently comparing these cell lines to Panc02.

Immunodeficient mice are used to generate xenograft models (Table 2). Immunodeficient mice include nude mice that lack T-cells, severe combined immunodeficient (SCID) mice that lack T and B cells, and nonobese diabetic (NOD)/SCID mice that lack T and B-cells, complement and have reduced NK cell activity. Nude mice are most often used. In these models, tumors are generated using human cancer cell lines or patient-derived xenografts (PDXs). The strength of using immunodeficient mice is the large variety of human cancer cell lines available. We and others have evaluated a variety of human colorectal and pancreatic cells lines. Interestingly, colorectal cancer cell lines with mutations in both *K-ras* and *p53*, such as the HCT116 subline 379.2, express higher levels of tissue factor (TF) than those with mutations in only *K-ras*, such as HCT116 [11]. There is also a wide range of TF expression in the different human pancreatic cell lines; HPAF-II, HPAC and BxPc-3 express high levels of TF, L3.6pl express medium levels, PANC-1 expresses low levels and MIAPaCa-2 do not express TF [12–14]. The A549 epithelial-like lung cancer cell line was isolated from human cancerous lung tissue [15, 16]. A weakness of using immunodeficient mice is that they are not able to mount a full immune response to the tumor.

Cancer cell lines may have a high or low metastatic potential in mice. It is simpler to use cancer cell lines that have a low metastatic potential for cancer-associated thrombosis studies because the tumor-burden is proportional to the size of the primary tumor and is not affected

by secondary metastasis. Highly metastatic tumors may also increase the death of mice (Hisada Y unpublished data).

Human and mouse cancer cell lines are easy to grow in culture. However, the gene expression pattern of these lines may be altered by maintaining the cells in culture. PDXs are considered superior to cancer cell lines because they maintain the pathology [17, 18], gene expression pattern [19] and single nucleotide polymorphisms [20] of primary tumors. PDXs are established by transferring them directly from the patient into mice and then maintained in immunodeficient mice [21]. Success rates of PDXs are variable (23–75%) and it can take 2–12 months to establish the different lines depending on the type of tumor [22]. The fundamental genotypic features of PDXs do not change over several passages in mice [23]. However, PDXs lose the gene expression profiles of primary tumors once the cells are cultured in vitro and these changes are irreversible [24]. The disadvantages of PDXs are that they must be maintained in mice, and it is difficult to transfer them between institutions [22].

Tumor site

There are two choices of site: subcutaneous or orthotopic (Tables 1 and 2). Subcutaneous tumors are typically implanted into the back or the right flank of mice between the dermis and underlying muscle. Matrigel is often used to support the establishment of the tumor. The subcutaneous tumor models are widely used because of the ease with which cells can be implanted and tumor size can be measured using calipers. The weakness of this model is that it does not reproduce the tumor microenvironment in patients [25]. In addition, tumors grown subcutaneously rarely metastasize even if the original tumor is metastatic [25].

Orthotopic tumors are more clinically relevant because the tumor microenvironment more closely resembles that found in patients [25]. Importantly, orthotopic tumors retain their capacity to metastasize. A disadvantage of this model is that it is more difficult to monitor tumor growth. This is important when performing thrombosis experiments because a defined size of tumor needs to be required to generate consistent results. To monitor tumor growth reporter genes, such as luciferase or green fluorescence protein (GFP), are introduced into the cell lines to allow non-invasive assessment of tumor size using luminescence or fluorescence molecular tomography. However, we have found that mice bearing orthotopic tumors are more prone to spontaneous or surgery-related death compared with mice bearing subcutaneous tumors (Hisada Y and Cooley BC, unpublished data).

As mentioned above, genetically engineered mice form spontaneous orthotopic tumors and are commercially available for most types of cancer. These models are more clinically relevant than xenograft models because they contain oncogenic mutations that occur in tumors in patients. They also mimic many features of tumor progression, such as angiogenesis, acquisition of secondary mutations and metastasis [26]. The disadvantage of genetically engineered mouse models is that tumor development is variable (typically 3 months or more), which means that it is not practical to compare the effect of similarly sized tumors on thrombosis.

Thrombosis models

There are several thrombosis models that have been used to study cancer-associated thrombosis in mice (Tables 1 and 2). The most popular are those involving the infrarenal vena cava (IVC). An advantage of the IVC is that high frequency ultrasonography can be used for non-invasive longitudinal monitoring of thrombus formation [14, 27, 28]. Clot formation is typically analyzed between 0–48 hours.

The IVC stasis model involves complete ligation of the IVC and side branches using non-reactive sutures [29]. Dorsal branches can also be cauterized to maximize clot formation [30]. The strengths of this model are that clot formation is close to 100% and large clots are formed (15–25 mg). This permits histological, cellular and molecular analysis of the clots to identify any changes that have been induced by the presence of the tumor. Weaknesses of this thrombosis model are that there is less blood flow to the injury site compared with the stenosis model and damage to the vessel wall exposes TF, which drives early clot formation and induces inflammation.

The IVC stenosis model is a modification of the St. Thomas model without the vessel injury step [31]. Typically, this involves formation of an area of ~90% stenosis by ligating of the IVC over a spacer with subsequent removal [32]. Some groups ligate the side and back branches [33]. Flow restriction appears to activate the endothelium and trigger clot formation, although a low level of vessel injury may also expose vessel wall TF [32]. The strength of this model is that it better mimics deep vein thrombosis in patients, although the clots form upstream of the stenotic site and grow against the direction of blood flow rather than originating in within valve pockets and growing in the direction of blood flow [31]. Weaknesses of the model are that the incidence of clot formation is quite variable and the clots are smaller (5–20 mg) compared to the stasis model and it is likely that complete occlusion will occur after the formation of a clot.

It is important to note that there are significant differences between the stasis and stenosis models in terms of factors that contribute to clot formation. For instance, vessel wall TF drives thrombosis in the IVC stasis model whereas leukocyte TF drives thrombosis in the stenosis model [34, 35]. In contrast, neutrophils and neutrophil extracellular traps (NETs) contribute to thrombosis in the IVC stenosis model but not in the stasis model [36]. Similarly, mice with a platelet-specific deletion of C-type lectin-like receptor 2 have reduced thrombosis in the stenosis but not the stasis model [37]. These differences should be considered when interpreting data from tumor-bearing mice.

Ferric chloride (FeCl_3) application is used to initiate thrombosis in large (IVC) and small (saphenous) veins, as well as the carotid artery and mesenteric microvessels [38, 39]. FeCl_3 induces oxidative damage to vessels with different concentrations and application times used to alter the severity of the model. Surprisingly, clots formed in the IVC exposed to FeCl_3 are quite small (~5 mg). For the saphenous vein and carotid artery models time to occlusion is measured. The strength of the FeCl_3 models are that they are both easy to perform and reproducible. However, the non-physiologic nature of the induction represents a significant weakness [31, 40].

The Rose Bengal photochemical model also generates free radicals that injure the inside of the vessel and time to occlusion is measured. The strengths of this model is that it is easy to conduct and reproducible. However, adjustments to the intensity of illumination, duration of illumination, and dose of dye are required depending on the type of vessel used [41, 42]. As with the FeCl_3 models the mechanism of vessel injury is not physiologic.

The laser induced-injury model generates clots on the inner surface of microvessels of the cremaster muscle that are analyzed in real-time by intravital microscopy. Clot formation is generally visualized by the accumulation of labeled antibodies against platelets and fibrin [43]. The strength of this model is that it captures real-time images of clot formation; the weakness is that it analyzes clot formation in small vessels that may not be relevant to deep vein thrombosis in cancer patients [44].

TF-positive microvesicles and cancer-associated thrombosis in mouse models

The TF/FVIIa complex is the primary activator of blood coagulation [45]. High levels of TF are expressed by a variety of tumors, particularly pancreatic tumors, as well as human and mouse pancreatic cancer cell lines [12–14, 46, 47]. In pancreatic cancer patients increased levels of microvesicle (MV) TF activity are associated with VTE [48, 49]. However, this association is not observed in patients with other types of cancer, such as gastric, brain, colorectal, ovarian or lung cancer [50–53] (unpublished data). These clinical studies have spurred a number of investigators to analyze the role of tumor-derived, TF+ MVs in mouse models of cancer-associated thrombosis.

The first series of studies reported increased levels of circulating tumor-derived, TF+ MVs and activation of coagulation in mice bearing human tumors. The first study used SCID mice with subcutaneous human colorectal tumors (HCT116) and found a significant correlation between circulating human TF antigen and the tumor volume [11]. Similar results were observed in nude mice bearing orthotopic human pancreatic tumors (L3.6pl) [12]. The authors reported that tumor weight was correlated with human TF antigen and activation of coagulation, measured by plasma levels of thrombin-antithrombin (TAT) complexes [12]. We found that nude mice bearing orthotopic human pancreatic tumors (HPAF-II) had increased human MV TF activity and TAT complex in their circulation [13]. In contrast, no TF MVs or activation of coagulation were observed when the HPAF-II tumors or two other human pancreatic cell lines (BxPc-3 and AsPc-1) were grown subcutaneously [13] (data not shown). In another study, we used the human pancreatic cell line BxPc-3 [14]. Similar to the studies with HPAF-II, we found that nude mice bearing orthotopic BxPc-3 tumors had increased levels of TAT complex [14, 28]. Importantly, administration of an anti-human TF monoclonal antibody abolished the increase in TAT complex [13]. These studies indicate that tumors release TF+ MVs into the circulation, and human TF expressed by the tumors is responsible for the activation of coagulation in the tumor-bearing mice.

The second series of studies measured the effect of different tumors on thrombosis in mice (Tables 1 and 2). One study used two TF expressing cell lines: the murine pancreatic cancer cell line Panc02 and the murine lung cancer cell line LLC [47]. C57BL/6 mice with

subcutaneous Panc02 tumors expressing GFP had detectable GFP-positive MVs in their circulation and these tumor-derived MVs accumulated at the site of FeCl₃-induced vessel injury [47]. Furthermore, mice bearing subcutaneous Panc02 or LLC tumors had shortened occlusion times after FeCl₃ injury of mesenteric vessels compared with controls [47]. Administration of an anti-P-selectin antibody reduced thrombosis in tumor-bearing mice [47]. Another study from the same group found that C57BL/6 mice bearing subcutaneous Panc02 had increased thrombosis in a laser-induced injury model of cremaster muscle microvessels compared to controls [54]. Finally, it was shown that C57BL/6 mice bearing subcutaneous Panc02 tumors had an increase in incidence of thrombosis and clot weight in the IVC stenosis model at 3 hours [55]. However, in contrast to the results with the mesenteric thrombosis model, the enhancement of thrombosis in the IVC stenosis model was not reduced by P-selectin deletion [55]. Further studies are needed to determine the pathways involved in the increased thrombosis in C57BL/6 mice with Panc02 pancreatic tumors.

We used a xenograft model to study the role of tumor-derived TF+ MVs in thrombosis (Table 2). In our initial study with nude mice bearing orthotopic HPAF-II tumors, we observed a shortening of the occlusion time in the FeCl₃ saphenous vein model [13]. However, we did not see any difference in clot weight in the IVC stenosis model at 3 hours between mice bearing orthotopic HPAF-II and control mice, although there was a low incidence of thrombosis [13]. In a second study, we found that nude mice bearing orthotopic BxPC-3 tumors had an increased clot area at 3, 6, 9 and 24 hours after IVC stenosis but the incidence of clots in tumor-bearing mice was not significantly increased compared to control mice [14]. Due to the variability of the IVC stenosis model, we changed to the IVC stasis model to study the role of tumor-derived TF+ MV in cancer-associated thrombosis [28]. In addition, we generated a new line of BxPc-3 that expresses luciferase to allow non-invasive monitoring of tumor size. Consistent with our previous results with showing that there was no increase in human TF+ MVs and TAT complex in mice with subcutaneous human pancreatic tumors. In addition, we did not observe an increase in clot weight in nude mice bearing either BxPc-3 or AsPc-1 subcutaneous tumors compared to clots in control mice (Figure 1). In contrast, we observed that nude mice bearing orthotopic BxPc-3 tumors had significantly larger clots at 48 hours compared with clots in control mice [28]. Analysis of the clot area at different time points revealed an increase in the clot area in tumor-bearing mice at 24 and 48 hours but not at 3 and 6 hours compared with mice without tumors [28]. We believe that at 3 and 6 hours clot formation is primarily driven by vessel wall TF exposed during the surgery. At 24 and 48 hours vessel wall TF no longer drives clot formation and this allows us to detect the contribution of circulating tumor-derived TF+ MV to clot formation. We observed human TF activity in clots from tumor-bearing mice indicating the incorporation of tumor-derived MVs into the clot [28]. Importantly, administration of an anti-human TF antibody abolished the enhancement of clot formation in tumor-bearing mice but did not affect the size of the clot in control mice [28]. These data indicate that tumor-derived, TF+ MVs enhance thrombosis in mice bearing orthotopic BxPc-3 tumors.

Neutrophil Extracellular Traps

NETs are released from activated neutrophils and contain granule proteins and chromatin. NETs were first described as a method of killing bacteria [56]. More recently, they were found in both venous and arterial clots, suggesting that they may contribute to thrombosis [57–59]. Indeed, mouse studies demonstrated that NETs contribute to thrombosis in the IVC stenosis model [33, 60]. In terms of cancer-associated thrombosis, plasma citrullinated histone H3, a marker of NETs, is associated with venous thrombosis in cancer patients [61]. Further, mouse studies sought to determine the role of NETs in cancer-associated thrombosis.

The murine mammary cancer cell line 4T1 has been used to study the role of NETs in mouse models of cancer-associated thrombosis. BALB/c mice with orthotopic 4T1 tumors had increased levels of granulocyte colony-stimulating factor (G-CSF), neutrophils, plasma DNA and citrullinated histone H3 compared to controls [62]. The presence of the tumor also induced a pro-thrombotic state in the mice with elevated levels of plasma von Willebrand factor, soluble P-selectin and fibrinogen [62]. Indeed, fibrin-rich thrombi were observed in veins of the lungs of tumor-bearing mice [62]. However, the study did not analyze the effect of tumors on thrombosis models. A second study also observed increased levels of neutrophils as well as plasma myeloperoxidase and DNA in mice with orthotopic 4T1 tumors [63]. We found increased plasma levels of G-CSF and neutrophils in nude mice with orthotopic BxPc-3 tumors [14] (unpublished data). These studies suggest that the presence of 4T1 and BxPc-3 tumors increases neutrophil levels and NET formation.

Leal and colleagues examined the effect of orthotopic 4T1 tumors on both arterial and venous thrombosis [63]. The presence of 4T1 tumors shortened the occlusion time in the FeCl₃ carotid artery model and a Rose Bengal/laser-induced jugular vein injury model [63]. Arterial thrombi from tumor-bearing mice contained Ly6G⁺ neutrophils, and extracellular fiber-like DNA consistent with the presence of NETs. Interestingly, administration of DNase I abolished the increase in venous thrombosis in tumor-bearing mice without affecting venous thrombosis in control mice [63]. In contrast, DNase I treatment prolonged the occlusion time in both control and tumor-bearing mice in FeCl₃ carotid artery model [63]. These studies suggest that NETs contribute to arterial and venous thrombosis in mice with orthotopic 4T1 tumors.

Other mouse models of cancer-associated thrombosis

One study analyzed thrombosis in BALB/c nude mice bearing subcutaneous human lung adenocarcinoma tumors (A549) [64]. Tumor-bearing mice had increased clot weights in an IVC stenosis model and shortened occlusion times in a FeCl₃ saphenous vein model [64]. Furthermore, it was found that the VEGF inhibitor bevacizumab increased the plasma concentration of tumor-derived PAI-1 and clot weight in the IVC stenosis model at 3 hours [64]. Bevacizumab also increased thrombosis in non-tumor bearing wild-type mice but not in PAI-1 deficient mice [64]. These studies indicate that the presence of subcutaneous A549 tumors increase thrombosis in mice.

Another study investigated the role of growth arrest-specific 6 in a mouse model of cancer-associated thrombosis using the mouse lung carcinoma (M27) and Eμ-myc B-cell lymphoma cell lines [65]. C57BL/6N mice bearing M27 lung tumors or Eμ-myc B-cell lymph node tumors had an increase in thrombus size in the FeCl₃ IVC model [65]. Thrombus size was measured by high frequency ultrasonography [65]. Interestingly, the clot size was smaller in growth arrest-specific 6 deficient mice and no increase was observed in the presence of tumors [65].

4. Summary

Different mouse models can be used to study cancer-associated thrombosis. The strengths of using immunocompetent mice are that they will have a full immune response to the tumor and gene knock-out mice can be used to study the role of different proteins in cancer-associated thrombosis. However, there are a limited number of mouse cancer lines available. In contrast, there are numerous human cancer cell lines and PDXs that can be used with immunodeficient mice. A weakness of immunodeficient mice is that they have an impaired immune response. Tumor growth can be more easily monitored in subcutaneous tumor compared to orthotopic tumors. However, a strength of using orthotopic tumors is that the tumor microenvironment is more similar to that found in patients. Although a variety of different thrombosis models have been used, the IVC stenosis or stasis are most common. Further studies are needed to determine the role of different pathways in thrombosis in mice bearing different types of tumors.

Acknowledgments

This work was supported by grants from the NIH (Y.H. T32 HL007149-41), the K. G. Jebsen Thrombosis Research and Expertise Center and the John C. Parker Professorship (NM). Brian C. Cooley and The Animal Surgery Core Laboratory of the McAllister Heart Institute at University of North Carolina at Chapel Hill performed the thrombosis experiments. We would like to thank Dr. Steven P. Grover and Reaves Houston for providing comments on the manuscript.

References

1. Pabinger I, Thaler J, Ay C. Biomarkers for prediction of venous thromboembolism in cancer. *Blood*. 2013; 122(12):2011–2018. [PubMed: 23908470]
2. Timp JF, Braekkan SK, Versteeg HH, Cannegieter SC. Epidemiology of cancer-associated venous thrombosis. *Blood*. 2013; 122(10):1712–1723. [PubMed: 23908465]
3. Stein PD, Beemath A, Meyers FA, Skaf E, Sanchez J, Olson RE. Incidence of venous thromboembolism in patients hospitalized with cancer. *Am J Med*. 2006; 119(1):60–68. [PubMed: 16431186]
4. Khorana AA, Kuderer NM, Culakova E, Lyman GH, Francis CW. Development and validation of a predictive model for chemotherapy-associated thrombosis. *Blood*. 2008; 111(10):4902–4907. [PubMed: 18216292]
5. Ay C, Dunkler D, Marosi C, Chiriac AL, Vormittag R, Simanek R, Quehenberger P, Zielinski C, Pabinger I. Prediction of venous thromboembolism in cancer patients. *Blood*. 2010; 116(24):5377–5382. [PubMed: 20829374]
6. Hisada Y, Mackman N. Cancer-associated pathways and biomarkers of venous thrombosis. *Blood*. 2017; 130(13):1499–1506. [PubMed: 28807983]
7. Aslakson CJ, Miller FR. Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor. *Cancer Res*. 1992; 52(6):1399–1405. [PubMed: 1540948]

8. Mayo JG. Biologic characterization of the subcutaneously implanted Lewis lung tumor. *Cancer Chemother Rep* 2. 1972; 3(1):325–330. [PubMed: 4660735]
9. Corbett TH, Roberts BJ, Leopold WR, Peckham JC, Wilkoff LJ, Griswold DP Jr, Schabel FM Jr. Induction and chemotherapeutic response of two transplantable ductal adenocarcinomas of the pancreas in C57BL/6 mice. *Cancer Res.* 1984; 44(2):717–726. [PubMed: 6692374]
10. Hingorani SR, Wang L, Multani AS, Combs C, Deramaudt TB, Hruban RH, Rustgi AK, Chang S, Tuveson DA. Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell.* 2005; 7(5):469–483. [PubMed: 15894267]
11. Yu JL, May L, Lhotak V, Shahrzad S, Shirasawa S, Weitz JI, Coomber BL, Mackman N, Rak JW. Oncogenic events regulate tissue factor expression in colorectal cancer cells: implications for tumor progression and angiogenesis. *Blood.* 2005; 105(4):1734–1741. [PubMed: 15494427]
12. Davila M, Amirkhosravi A, Coll E, Desai H, Robles L, Colon J, Baker CH, Francis JL. Tissue factor-bearing microparticles derived from tumor cells: impact on coagulation activation. *J Thromb Haemost.* 2008; 6(9):1517–1524. [PubMed: 18433463]
13. Wang JG, Geddings JE, Aleman MM, Cardenas JC, Chantrathammachart P, Williams JC, Kirchhofer D, Bogdanov VY, Bach RR, Rak J, Church FC, Wolberg AS, Pawlinski R, Key NS, Yeh JJ, Mackman N. Tumor-derived tissue factor activates coagulation and enhances thrombosis in a mouse xenograft model of human pancreatic cancer. *Blood.* 2012; 119(23):5543–5552. [PubMed: 22547577]
14. Geddings JE, Hisada Y, Boulaftali Y, Getz TM, Whelihan M, Fuentes R, Dee R, Cooley BC, Key NS, Wolberg AS, Bergmeier W, Mackman N. Tissue factor-positive tumor microvesicles activate platelets and enhance thrombosis in mice. *J Thromb Haemost.* 2016; 14(1):153–166. [PubMed: 26516108]
15. Giard DJ, Aaronson SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H, Parks WP. In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. *J Natl Cancer Inst.* 1973; 51(5):1417–1423. [PubMed: 4357758]
16. Lieber M, Smith B, Szakal A, Nelson-Rees W, Todaro G. A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. *Int J Cancer.* 1976; 17(1):62–70. [PubMed: 175022]
17. Loukopoulou P, Kanetaka K, Takamura M, Shibata T, Sakamoto M, Hirohashi S. Orthotopic transplantation models of pancreatic adenocarcinoma derived from cell lines and primary tumors and displaying varying metastatic activity. *Pancreas.* 2004; 29(3):193–203. [PubMed: 15367885]
18. DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, Ebbert MT, Factor R, Matsen C, Milash BA, Nelson E, Neumayer L, Randall RL, Stijleman IJ, Welm BE, Welm AL. Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. *Nat Med.* 2011; 17(11):1514–1520. [PubMed: 22019887]
19. Zhao X, Liu Z, Yu L, Zhang Y, Baxter P, Voicu H, Gurusiddappa S, Luan J, Su JM, Leung HC, Li XN. Global gene expression profiling confirms the molecular fidelity of primary tumor-based orthotopic xenograft mouse models of medulloblastoma. *Neuro Oncol.* 2012; 14(5):574–583. [PubMed: 22459127]
20. McEvoy J, Ulyanov A, Brennan R, Wu G, Pounds S, Zhang J, Dyer MA. Analysis of MDM2 and MDM4 single nucleotide polymorphisms, mRNA splicing and protein expression in retinoblastoma. *PLoS One.* 2012; 7(8):e42739. [PubMed: 22916154]
21. Morton CL, Houghton PJ. Establishment of human tumor xenografts in immunodeficient mice. *Nat Protoc.* 2007; 2(2):247–250. [PubMed: 17406581]
22. Siolas D, Hannon GJ. Patient-derived tumor xenografts: transforming clinical samples into mouse models. *Cancer Res.* 2013; 73(17):5315–5319. [PubMed: 23733750]
23. Rubio-Viqueira B, Jimeno A, Cusatis G, Zhang X, Iacobuzio-Donahue C, Karikari C, Shi C, Danenberg K, Danenberg PV, Kuramochi H, Tanaka K, Singh S, Salimi-Moosavi H, Bouraoud N, Amador ML, Altiock S, Kulesza P, Yeo C, Messersmith W, Eshleman J, Hruban RH, Maitra A, Hidalgo M. An in vivo platform for translational drug development in pancreatic cancer. *Clin Cancer Res.* 2006; 12(15):4652–4661. [PubMed: 16899615]

24. Daniel VC, Marchionni L, Hierman JS, Rhodes JT, Devereux WL, Rudin CM, Yung R, Parmigiani G, Dorsch M, Peacock CD, Watkins DN. A primary xenograft model of small-cell lung cancer reveals irreversible changes in gene expression imposed by culture in vitro. *Cancer Res.* 2009; 69(8):3364–3373. [PubMed: 19351829]
25. Hoffman RM. Patient-derived orthotopic xenografts: better mimic of metastasis than subcutaneous xenografts. *Nat Rev Cancer.* 2015; 15(8):451–452. [PubMed: 26422835]
26. Politi K, Pao W. How genetically engineered mouse tumor models provide insights into human cancers. *J Clin Oncol.* 2011; 29(16):2273–2281. [PubMed: 21263096]
27. Geddings J, Aleman MM, Wolberg A, von Bruhl ML, Massberg S, Mackman N. Strengths and weaknesses of a new mouse model of thrombosis induced by inferior vena cava stenosis: communication from the SSC of the ISTH. *J Thromb Haemost.* 2014; 12(4):571–573. [PubMed: 24460606]
28. Hisada Y, Ay C, Auriemma AC, Cooley BC, Mackman N. Human pancreatic tumors grown in mice release tissue factor-positive microvesicles that increase venous clot size. *J Thromb Haemost.* 2017; 15(11):2208–2217. [PubMed: 28834179]
29. Myers D Jr, Farris D, Hawley A, Wroblewski S, Chapman A, Stoolman L, Knibbs R, Strieter R, Wakefield T. Selectins influence thrombosis in a mouse model of experimental deep venous thrombosis. *J Surg Res.* 2002; 108(2):212–221. [PubMed: 12505044]
30. Diaz JA, Ballard-Lipka NE, Farris DM, Hawley AE, Wroblewski SK, Myers DD, Henke PK, Lawrence DA, Wakefield TW. Impaired fibrinolytic system in ApoE gene-deleted mice with hyperlipidemia augments deep vein thrombosis. *J Vasc Surg.* 2012; 55(3):815–822. [PubMed: 22119245]
31. Diaz JA, Obi AT, Myers DD Jr, Wroblewski SK, Henke PK, Mackman N, Wakefield TW. Critical review of mouse models of venous thrombosis. *Arterioscler Thromb Vasc Biol.* 2012; 32(3):556–562. [PubMed: 22345593]
32. Brill A, Fuchs TA, Chauhan AK, Yang JJ, De Meyer SF, Kollnberger M, Wakefield TW, Lammle B, Massberg S, Wagner DD. von Willebrand factor-mediated platelet adhesion is critical for deep vein thrombosis in mouse models. *Blood.* 2011; 117(4):1400–1407. [PubMed: 20959603]
33. Brill A, Fuchs TA, Savchenko AS, Thomas GM, Martinod K, De Meyer SF, Bhandari AA, Wagner DD. Neutrophil extracellular traps promote deep vein thrombosis in mice. *J Thromb Haemost.* 2012; 10(1):136–144. [PubMed: 22044575]
34. Day SM, Reeve JL, Pedersen B, Farris DM, Myers DD, Im M, Wakefield TW, Mackman N, Fay WP. Macrovascular thrombosis is driven by tissue factor derived primarily from the blood vessel wall. *Blood.* 2005; 105(1):192–198. [PubMed: 15339841]
35. von Bruhl ML, Stark K, Steinhart A, Chandraratne S, Konrad I, Lorenz M, Khandoga A, Tirniceriu A, Coletti R, Kollnberger M, Byrne RA, Laitinen I, Walch A, Brill A, Pfeiler S, Manukyan D, Braun S, Lange P, Riegger J, Ware J, Eckart A, Haidari S, Rudelius M, Schulz C, Ehtler K, Brinkmann V, Schwaiger M, Preissner KT, Wagner DD, Mackman N, Engelmann B, Massberg S. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. *J Exp Med.* 2012; 209(4):819–835. [PubMed: 22451716]
36. El-Sayed OM, Dewyer NA, Luke CE, Elflin M, Laser A, Hogaboam C, Kunkel SL, Henke PK. Intact Toll-like receptor 9 signaling in neutrophils modulates normal thrombogenesis in mice. *J Vasc Surg.* 2016; 64(5):1450–1458. e1451. [PubMed: 26482993]
37. Payne H, Ponomarev T, Watson SP, Brill A. Mice with a deficiency in CLEC-2 are protected against deep vein thrombosis. *Blood.* 2017; 129(14):2013–2020. [PubMed: 28104688]
38. Chauhan AK, Kisucka J, Lamb CB, Bergmeier W, Wagner DD. von Willebrand factor and factor VIII are independently required to form stable occlusive thrombi in injured veins. *Blood.* 2007; 109(6):2424–2429. [PubMed: 17119108]
39. Buyue Y, Whinna HC, Sheehan JP. The heparin-binding exosite of factor IXa is a critical regulator of plasma thrombin generation and venous thrombosis. *Blood.* 2008; 112(8):3234–3241. [PubMed: 18647957]
40. Ciciliano JC, Sakurai Y, Myers DR, Fay ME, Hechler B, Meeks S, Li R, Dixon JB, Lyon LA, Gachet C, Lam WA. Resolving the multifaceted mechanisms of the ferric chloride thrombosis

- model using an interdisciplinary microfluidic approach. *Blood*. 2015; 126(6):817–824. [PubMed: 25931587]
41. Perez P, Alarcon M, Fuentes E, Palomo I. Thrombus formation induced by laser in a mouse model. *Exp Ther Med*. 2014; 8(1):64–68. [PubMed: 24944598]
 42. Rauova L. "Radical" model of thrombosis. *Blood*. 2012; 119(8):1798–1799. [PubMed: 22361971]
 43. Falati S, Gross P, Merrill-Skoloff G, Furie BC, Furie B. Real-time in vivo imaging of platelets, tissue factor and fibrin during arterial thrombus formation in the mouse. *Nat Med*. 2002; 8(10): 1175–1181. [PubMed: 12244306]
 44. Vogel A, Venugopalan V. Mechanisms of pulsed laser ablation of biological tissues. *Chem Rev*. 2003; 103(2):577–644. [PubMed: 12580643]
 45. Mackman N. Role of tissue factor in hemostasis and thrombosis. *Blood Cells Mol Dis*. 2006; 36(2):104–107. [PubMed: 16466951]
 46. Khorana AA, Ahrendt SA, Ryan CK, Francis CW, Hruban RH, Hu YC, Hostetter G, Harvey J, Taubman MB. Tissue factor expression, angiogenesis, and thrombosis in pancreatic cancer. *Clin Cancer Res*. 2007; 13(10):2870–2875. [PubMed: 17504985]
 47. Thomas GM, Panicot-Dubois L, Lacroix R, Dignat-George F, Lombardo D, Dubois C. Cancer cell-derived microparticles bearing P-selectin glycoprotein ligand 1 accelerate thrombus formation in vivo. *J Exp Med*. 2009; 206(9):1913–1927. [PubMed: 19667060]
 48. Khorana AA, Francis CW, Menzies KE, Wang JG, Hyrien O, Hathcock J, Mackman N, Taubman MB. Plasma tissue factor may be predictive of venous thromboembolism in pancreatic cancer. *J Thromb Haemost*. 2008; 6(11):1983–1985. [PubMed: 18795992]
 49. Tesselaar ME, Romijn FP, van der Linden IK, Bertina RM, Osanto S. Microparticle-associated tissue factor activity in cancer patients with and without thrombosis. *J Thromb Haemost*. 2009; 7(8):1421–1423. [PubMed: 19500241]
 50. Thaler J, Ay C, Mackman N, Bertina RM, Kaider A, Marosi C, Key NS, Barcel DA, Scheithauer W, Kornek G, Zielinski C, Pabinger I. Microparticle-associated tissue factor activity, venous thromboembolism and mortality in pancreatic, gastric, colorectal and brain cancer patients. *J Thromb Haemost*. 2012; 10(7):1363–1370. [PubMed: 22520016]
 51. van Doormaal F, Kleinjan A, Berckmans RJ, Mackman N, Manly D, Kamphuisen PW, Richel DJ, Buller HR, Sturk A, Nieuwland R. Coagulation activation and microparticle-associated coagulant activity in cancer patients. An exploratory prospective study. *Thromb Haemost*. 2012; 108(1):160–165. [PubMed: 22535219]
 52. Bharthuar A, Khorana AA, Hutson A, Wang JG, Key NS, Mackman N, Iyer RV. Circulating microparticle tissue factor, thromboembolism and survival in pancreaticobiliary cancers. *Thromb Res*. 2013; 132(2):180–184. [PubMed: 23856554]
 53. Cohen JG, Prendergast E, Geddings JE, Walts AE, Agadjanian H, Hisada Y, Karlan BY, Mackman N, Walsh CS. Evaluation of venous thrombosis and tissue factor in epithelial ovarian cancer. *Gynecol Oncol*. 2017; 146(1):146–152. [PubMed: 28501328]
 54. Mezouar S, Darbousset R, Dignat-George F, Panicot-Dubois L, Dubois C. Inhibition of platelet activation prevents the P-selectin and integrin-dependent accumulation of cancer cell microparticles and reduces tumor growth and metastasis in vivo. *Int J Cancer*. 2015; 136(2):462–475. [PubMed: 24889539]
 55. Thomas GM, Brill A, Mezouar S, Crescence L, Gallant M, Dubois C, Wagner DD. Tissue factor expressed by circulating cancer cell-derived microparticles drastically increases the incidence of deep vein thrombosis in mice. *J Thromb Haemost*. 2015; 13(7):1310–1319. [PubMed: 25955268]
 56. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. *Science*. 2004; 303(5663):1532–1535. [PubMed: 15001782]
 57. Fuchs TA, Brill A, Duerschmied D, Schatzberg D, Monestier M, Myers DD Jr, Wroblewski SK, Wakefield TW, Hartwig JH, Wagner DD. Extracellular DNA traps promote thrombosis. *Proc Natl Acad Sci U S A*. 2010; 107(36):15880–15885. [PubMed: 20798043]
 58. Longstaff C, Varju I, Sotonyi P, Szabo L, Krumrey M, Hoell A, Bota A, Varga Z, Komorowicz E, Kolev K. Mechanical stability and fibrinolytic resistance of clots containing fibrin, DNA, and histones. *J Biol Chem*. 2013; 288(10):6946–6956. [PubMed: 23293023]

59. Mangold A, Alias S, Scherz T, Hofbauer T, Jakowitsch J, Panzenbock A, Simon D, Laimer D, Bangert C, Kammerlander A, Mascherbauer J, Winter MP, Distelmaier K, Adlbrecht C, Preissner KT, Lang IM. Coronary neutrophil extracellular trap burden and deoxyribonuclease activity in ST-elevation acute coronary syndrome are predictors of ST-segment resolution and infarct size. *Circ Res.* 2015; 116(7):1182–1192. [PubMed: 25547404]
60. Martinod K, Demers M, Fuchs TA, Wong SL, Brill A, Gallant M, Hu J, Wang Y, Wagner DD. Neutrophil histone modification by peptidylarginine deiminase 4 is critical for deep vein thrombosis in mice. *Proc Natl Acad Sci U S A.* 2013; 110(21):8674–8679. [PubMed: 23650392]
61. Mauracher L-M, Thaler J, Posch F, Martinod K, Grilz E, Daullary T, Hell L, Kober S, Brostjan C, Zielinski C, Ay C, Wagner DD, Pabinger I. Citrullinated Histone H3, a Biomarker of Neutrophil Extracellular Trap Formation, is Associated with Increased Risk of Venous Thromboembolism in Patients with Cancer. *Res Pract Thromb Haemost.* 2017; 1(Suppl 1):49.
62. Demers M, Krause DS, Schatzberg D, Martinod K, Voorhees JR, Fuchs TA, Scadden DT, Wagner DD. Cancers predispose neutrophils to release extracellular DNA traps that contribute to cancer-associated thrombosis. *Proc Natl Acad Sci U S A.* 2012; 109(32):13076–13081. [PubMed: 22826226]
63. Leal AC, Mizurini DM, Gomes T, Rochael NC, Saraiva EM, Dias MS, Werneck CC, Sielski MS, Vicente CP, Monteiro RQ. Tumor-Derived Exosomes Induce the Formation of Neutrophil Extracellular Traps: Implications For The Establishment of Cancer-Associated Thrombosis. *Sci Rep.* 2017; 7(1):6438. [PubMed: 28743887]
64. Chen N, Ren M, Li R, Deng X, Li Y, Yan K, Xiao L, Yang Y, Wang L, Luo M, Fay WP, Wu J. Bevacizumab promotes venous thromboembolism through the induction of PAI-1 in a mouse xenograft model of human lung carcinoma. *Mol Cancer.* 2015; 14:140. [PubMed: 26215730]
65. Aghourian MN, Lemarie CA, Bertin FR, Blostein MD. Prostaglandin E synthase is upregulated by Gas6 during cancer-induced venous thrombosis. *Blood.* 2016; 127(6):769–777. [PubMed: 26585956]

Figure 1. Clots from subcutaneous tumor-bearing mice

BxPc-3 and AsPc-1 subcutaneous tumors were grown to 0.68 g [0.50–1.08] and 1.01 g [0.65–1.29], respectively (mean weight [range]). Clots were harvested and weighed at 48 hours after IVC stasis. Data are shown as mean $\pm$ standard deviation. Data were analyzed one way ANOVA with Dunnett's test.

Table 1

Summary of allograft models of cancer-associated thrombosis.

Mouse	Cancer cell	Tumor site	Thrombosis model	Blood vessel	Observation	Ref
C57BL/6	Panc02, LLC1	s.c.	FeCl ₃	mesenteric venules	Reduced time to occlusion	47
C57BL/6	Panc02	s.c.	Laser-induced injury FeCl ₃	mesenteric venules	Tumor-derived MVs accumulated at the site of injury	47
C57BL/6	Panc02	s.c.	Laser-induced injury	cremaster muscle microvessels	Tumor derived MV accumulation at the site of injury	54
C57BL/6	Panc02	s.c.	IVC stenosis	IVC	Increased incidence and thrombus weight	55
C57BL/6	M27 E _μ -myc	orthotopic	FeCl ₃	IVC	Gas6 deficient mice had reduced thrombus size compared with controls	65
BALB/c	4T1	orthotopic	FeCl ₃	carotid artery	Reduced time to occlusion. DNase I abolished enhanced thrombosis.	63
BALB/c	4T1	orthotopic	Rose Bengal	jugular vein	Reduced time to occlusion. DNase I reduced thrombus size	63

IVC; infrarenal vena cava, s.c.; subcutaneous

Table 2

Summary of xenograft models of cancer-associated thrombosis.

Mouse	Cancer cell	Tumor site	Thrombosis model	Blood vessel	Observation	Ref
Nude	HPAF II	orthotopic	IVC stenosis	IVC	No enhancement of thrombosis	13
Nude	HPAF II	orthotopic	FeCl ₃	saphenous vein	Shortened time to occlusion	13
Nude	BxPc-3	orthotopic	IVC stenosis	IVC	Increased thrombus area but no increase in incidence of thrombosis	14
Nude	BxPc-3	orthotopic	IVC stasis	IVC	Increased thrombus area and weight	28
Nude	A549	orthotopic	IVC stenosis	IVC	Increased thrombus weight	64
Nude	A549	orthotopic	FeCl ₃	saphenous vein	Reduced time to occlusion	64

IVC: infrarenal vena cava