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Cancer and platelet crosstalk: opportunities and challenges for aspirin and other antiplatelet agents

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Platelets have long been recognized as key players in hemostasis and thrombosis; however, growing evidence suggests that they are also significantly involved in cancer, the second leading cause of mortality worldwide. Preclinical and clinical studies showed that tumorigenesis and metastasis can be promoted by platelets through a wide variety of crosstalk between platelets and cancer cells. For example, cancer changes platelet behavior by directly inducing tumor-platelet aggregates, triggering platelet granule and extracellular vesicle release, altering platelet phenotype and platelet RNA profiles, and enhancing thrombopoiesis. Reciprocally, platelets reinforce tumor growth with proliferation signals, antiapoptotic effect, and angiogenic factors. Platelets also activate tumor invasion and sustain metastasis via inducing an invasive epithelial-mesenchymal transition phenotype of tumor cells, promoting tumor survival in circulation, tumor arrest at the endothelium, and extravasation. Furthermore, platelets assist tumors in evading immune destruction. Hence, cancer cells and platelets maintain a complex, bidirectional communication. Recently, aspirin (acetylsalicylic acid) has been recognized as a promising cancer-preventive agent. It is recommended at daily low dose by the US Preventive Services Task Force for primary prevention of colorectal cancer. The exact mechanisms of action of aspirin in chemoprevention are not very clear, but evidence has emerged that suggests a platelet-mediated effect. In this article, we will introduce how cancer changes platelets to be more cancer-friendly and highlight advances in the modes of action for aspirin in cancer prevention. We also discuss the opportunities, challenges, and opposing viewpoints on applying aspirin and other antiplatelet agents for cancer prevention and treatment. (*Blood*. 2018;131(16):1777-1789)

Introduction

Despite considerable progress in developing new approaches for cancer treatment over the past 2 decades, cancer continues to be an enormous challenge for public health. It is the second leading cause of mortality worldwide, and has overtaken cardiovascular diseases (CVDs) as the principal cause of death in United States.[1,2] Aspirin, a widely used antiplatelet and anti-inflammatory agent, has emerged as perhaps the most promising drug for cancer prevention.[3-5] Platelets are small anucleate blood cells generated from megakaryocytes in the bone marrow and also likely the lung.[6,7] Since the late 1960s, scientists and clinicians have begun to notice the links between platelets and cancer.[8] It has now become clearer that cancer cells can induce abnormalities in platelet number and function. In turn, platelets can promote tumor growth and metastasis.[6,9-15]

Overview of platelet functions

Platelets are key players in hemostasis and thrombosis, including those in tumor vasculature.[16-18] At sites of vascular injury, platelet adhesion, activation and aggregation, and elaboration of procoagulant surface activity,[19] are critical events to stop bleeding.[20-22] Low platelet counts in blood, such as immune-mediated and chemotherapy-induced thrombocytopenias,[23-27] may cause life-threatening bleeding.[28-30] However, improper platelet activation and aggregation may result in thrombosis, leading to CVD.[16,31-33] Importantly, 10% to 15% of cancer patients develop a cancer-associated thrombosis, especially venous thromboembolism, which is the second leading cause of death in cancer patients.[10,34] Tumor-activated platelets likely contribute to these thrombotic events.[35-38]

Numerous studies have investigated the molecular basis in mediating thrombosis.[16,39-41] Although fibrinogen has been documented to be required for platelet aggregation, recent evidence demonstrated that fibrinogen-independent platelet aggregation occurs in both animals[42-45] and humans.[46-48] Although proteins supporting this novel aggregation pathway remain to be studied, platelet αIIbβ3 integrin is essential,[44] and several αIIbβ3 ligands such as fibronectin, thrombospondin-1, and counter-receptor cadherin 6 may be involved.[21,39,49-52] These platelet receptors and their ligands also likely contribute to platelet–tumor interactions, metastasis, and cancer-associated thrombosis.

Emerging evidence indicates that platelets are versatile cells[6,53,54] involved in many other pathophysiological processes, such as

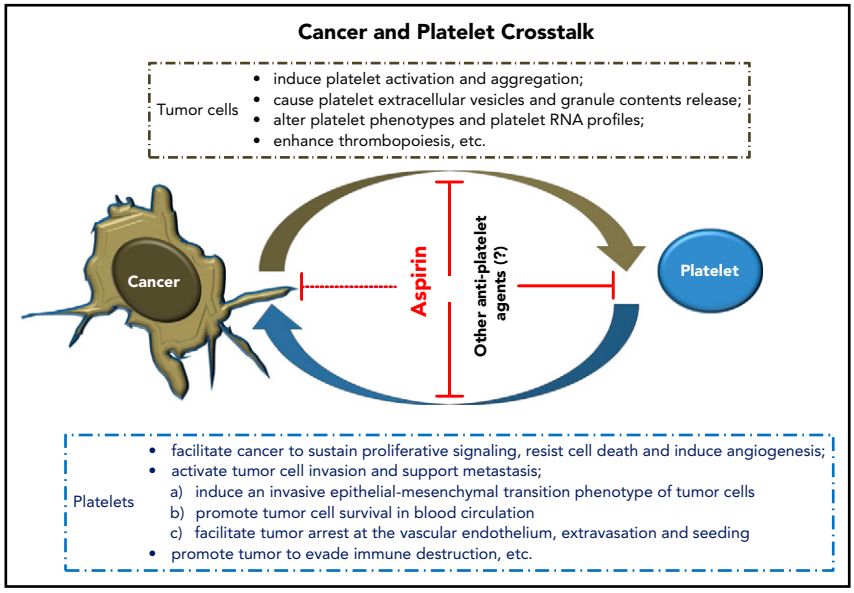

Figure 1. Cancer and platelet crosstalk.

immune responses,[55,56] angiogenesis,[57,58] and lymphatic vessel development.[59,60] These characteristics may affect their roles in cancer.

Reciprocal crosstalk between cancer and platelets

Elegant reviews have summarized the hallmarks of tumor cells acquired during their development, which control the transformation of normal cells to cancer (supplemental Data, available on the *Blood* Web site).[61,62] Notably, cancer can "dictate" platelets to support these key processes. We now know that tumor cells and platelets maintain a complex, bidirectional interaction in the blood and tumor microenvironment (TME) (Figure 1), although further investigation of details related to these interactions is required.

How cancer changes platelets

The concept of "tumor-educated platelets" (TEPs) is emerging and has been used by several groups.[10,63-66] Studies have shown that tumor cells can change platelet behaviors via several mechanisms.

TCIPA and formation of tumor-platelet aggregates The concept of tumor cell–induced platelet aggregation (TCIPA) can be traced back to the first observation in the late 19th century.[67] Despite the incompletely understood mechanism, which may vary depending on tumor type, platelet agonists (eg, thrombin, adenosine 5′-diphosphate) generated by the tumor cells and microenvironment seem to be the stimulators,[68] followed by the interactions of various platelet receptors and ligands. It is currently unknown how many of these receptors (supplemental Data)[52,69-78] are involved in TCIPA (ie, platelet–platelet, platelet–tumor, tumor–platelet–leukocyte aggregation[11,12]) and how they contribute to this process.

Several platelet receptors and their ligands, however, have been recently elucidated in TCIPA (Table 1). Platelet αIIbβ3, through binding fibrin(ogen) or fibrin–fibronectin complexes,[50,51] bridges tumor αVβ3.[79-81] Fibrin can be generated by the tumor cell tissue factor–initiated coagulation pathway. Platelet α6β1, through binding ADAM9 on tumor cells, enhances platelet activation and tumor cell extravasation.[82] Binding of platelet P-selectin to tumor P-selectin ligands[83-85] also mediates platelet–tumor cell microthrombi.[86-88] Platelet Toll-like receptor (TLR) 4 promotes TCIPA and metastasis through interaction with tumor-released high-mobility group box 1 protein.[89] In addition, platelet CLEC-2 induces TCIPA and thrombosis in tumor vessels and facilitates metastasis via ligation with tumor podoplanin.[71,72] High podoplanin expression on brain tumors was correlated with increased platelet aggregation and risk of venous thromboembolism in patients.[90]

Studies on GPIb-IX-V complex, however, have inconsistent findings. GPIbα knockout mice showed reduced lung metastasis, indicating its supportive roles.[91] Notably, de novo expression of von Willebrand factor (VWF) was also found in cancer cells of non-endothelial origin.[92] Thus, platelet GPIbα likely binds to tumor VWF and mediates TCIPA and metastasis. It will be worthwhile to test whether Anfibatide, a new anti-GPIbα polypeptide isolated from snake venom, could reduce metastasis.[93] Interestingly, a study also reported that a monoclonal antibody against GPIbα promoted melanoma metastasis.[94] One cannot exclude that some anti-GPIbα antibodies may activate platelets,[95-98] enhance TCIPA, and facilitate the observed metastasis.[94] It is necessary to investigate whether these confounding effects resulted from the use of different animal models and are reproducible in other tumor cell lines by different anti-GPIbα antibodies. This information is important for the further development of antiplatelet drugs targeting GPIbα[99] to control CVD and cancer.

Altogether, these studies demonstrate that tumor cells can activate platelets and induce TCIPA. There is no doubt that more platelet receptors and ligands will be identified in this process. Although TCIPA is not easily detected as a biomarker for cancer diagnosis and prognosis because of its relatively low frequency in peripheral blood, targeting these platelet receptor ligands may have great potential for new adjuvant antitumor therapies (Table 1).

Tumor cells induce platelet extracellular vesicle generation, granule release, and phenotype changes Following activation, aggregation with tumor cells and exposure to shear stress,

Table 1. Other antiplatelet agents that may affect tumor metastasis and tumorigenesis

Category	Agents	Targeting receptor–ligand interactions	Reported tumor types	Comments	References
Targeting direct molecule contacts between platelets and tumor cells					
αIIbβ3 integrin antagonists	Abciximab,* eptifibatide,* tirofiban,* RUC-4†; mAb10E5 and XV454,† anti-integrin PSI domain mAb†	Platelet αIIbβ3 integrin–plasma fibrin(ogen) or fibrin–fibronectin complex–tumor αVβ3 integrin	Melanoma, cancers of breast and likely prostate, pancreatic, ovarian, cervical; glioblastoma	αIIbβ3 is critical for bone metastasis of melanoma; activation of integrin controls metastasis in human breast cancer	79-81
α6β1 integrin antagonists	Anti–α6-antibody GoH3†	Platelet α6β1integrin–tumor ADAM9	Cancers of breast and colon	α6β1 promotes spontaneous and experimental lung metastasis	82
GPIbα inhibitors	Anfibatide,‡ H6B4,† NIT family mAb†	Platelet GPIbα–tumor VWF, (sub)endothelial VWF or P-selectin, leukocyte αMβ2	Melanoma	GPIbα supports experimental lung metastasis; anti-GPIbα inhibits interactions with VWF and thrombin, which may inhibit TCIPA and tumor arrest; anti-GPIbα decreases thrombopoietin generation and may inhibit tumor-induced thrombocytosis	91,93,119
TLR4 inhibition	Anti-HMGB1†	Platelet TLR-4–tumor HMGB1	Melanoma and lung cancer	TLR4 mediates tumor-induced platelet activation, tumor-platelet adhesion, and metastasis	89
P-selectin inhibitors	Anti–P-selectin antibody,† anti-CD24 (P-selectin ligand) antibody FL80†	Platelet P-selectin–tumor P-selectin ligands	Mucin-type ligands bearing sialyl-Lewis X on colon, prostate, small-cell lung cancers, and neuroblastoma; sulfated galactosylceramide-type ligands on colon cancer	P-selectin mediates tumor growth, metastasis, and platelet–tumor cell microthrombi	83-87
CLEC-2 inhibitors	Anti-mouse CLEC-2 mAb 2A2B10†	Platelet CLEC-2–tumor podoplanin	Melanoma, brain tumors, and likely squamous cell carcinoma of the lung, head, and neck	CLEC-2 promotes hematogenous tumor metastasis and prothrombotic state; high podoplanin induces platelet aggregation, correlates with increased risk of venous thromboembolism	71,72,90
GPVI antagonists	Revacept,‡ losartan,† scFv 9012†	Platelet GPVI–tumor fibrin(ogen) and/or subendothelial collagen	Melanoma and lung cancer	GPVI deficiency is associated with a 50% reduction in experimental lung metastasis	238-240
CD36 inhibitors	Anti-CD36 neutralizing antibody†	Platelet CD36–platelet released TSP-1–cancer cell CD36/integrins (?)	Oral squamous cell carcinoma, melanoma, breast cancer, etc	Anti-CD36 results in an antimetastatic effect; may inhibit CD36-mediated platelet activation and TCIPA	21,74-76,157
Category	**Agents**	**Comments**			**References**
Targeting platelet activation pathways					
P2Y12 antagonists	Clopidogrel,* prasugrel,* ticagrelor,* cangrelor*	Coadministration of clopidogrel with aspirin markedly improves the efficacy of adoptive T-cell therapy of cancer in animal models and prevents chronic hepatitis B–associated hepatocellular carcinoma. P2Y12 deficiency results in >85% reduction in the growth of syngeneic ovarian cancer tumors; ticagrelor reduces tumor growth by 75% compared with placebo			194,225,236
PAR1 antagonists	Vorapaxar,* atopaxar‡	Targeting PAR-1 is important for thrombin-enhanced metastasis			235
EP3 receptor antagonists	DG-041‡	DG-041 inhibits PGE2-dependent platelet activation and aggregation and prevents platelet-mediated induction of EMT in CRC			149,243

mAb, monoclonal antibody; PGE, prostaglandin E2; PSI, plexin-semaphorin-integrin.

*US Food and Drug Administration approved.

†Preclinical stage of development.

‡Phase 2.

platelets release extracellular vesicles (EVs), such as exosomes and microparticles.[100] Aggressive tumors are correlated with higher levels of platelet microparticles.[101,102] It has been shown that microRNA-223 delivered by platelet-derived microparticles is significantly increased in patients with non–small cell lung cancer (NSCLC).[103] Tumors also induce platelet granule release[104] and phenotype changes in cancer patients by increasing the secretion of pro-angiogenic proteins (see "Platelets facilitate cancer to sustain proliferative signaling, resist cell death, and induce tumor angiogenesis"), such as vascular endothelial growth factor (VEGF).[105] These cancer-associated features may be developed as early biomarkers for cancer screening.

Tumor cells alter platelet RNA profiles It has recently been highlighted that tumors can also alter platelet RNA profiles.[63,65,106-108] The exact mechanisms of RNA signature in TEP are not well understood. One mechanism might be via cancer cells releasing RNA into their local environment, likely through EV such as tumor-derived exosomes,[109-111] and transferring mutant RNA into platelets.[63,65,107,108] Indeed, platelets from cancer patients contained tumor-associated RNA biomarkers, such as EGFRvIII and PCA3 for glioma and prostate cancer, respectively.[107] Although it is not clear how tumor-derived exosome uptake by platelets occurs, plasma membrane fusion, clathrin-mediated endocytosis, and phagocytosis may be involved.[109] Importantly, messenger RNA (mRNA) sequencing of TEP can identify cancer patients with 96% accuracy and distinguish 6 primary tumor types, including NSCLC, glioblastoma, colorectal, pancreatic, hepatobiliary, and breast cancers with 71% accuracy.[63] In addition, TEP accurately detected both early- and late-stage NSCLC.[65] Because platelets are also anucleate cells and are easily isolated, this TEP-based RNA biosource, despite requiring further characterization, may serve as an attractive platform for liquid biopsy, which is a primarily blood-based, minimally invasive assay for cancer diagnosis, prognosis, and treatment monitoring in the context of precision medicine.[112]

Tumor cells enhance thrombopoiesis The extent of thrombocytosis has a close relationship with the poor clinical outcome for the majority of malignancies, such as cancers of ovary, bladder, kidney, pancreas, esophagogastric, uterus, and, in particular, colorectal and lung.[113,114] Thrombocytosis in primary care is also positively correlated with an increased risk of certain cancers.[115] Evidence has shown that the increased thrombopoietic cytokine production by tumor and host tissues, such as interleukin-1 (IL-1), IL-3, IL-11, and particularly tumor-derived IL-6, is the predominant cause of hepatic thrombopoietin generation and thrombocytosis.[116,117] Tumor-derived platelet factor 4 (PF4) has also been reported to promote platelet production.[118] Intriguingly, we recently found that platelet GPIbα is required for platelet-induced hepatic thrombopoietin generation in humans and mice.[119] It is currently unknown whether these tumor-released cytokines and platelet GPIbα can synergistically trigger thrombopoietin production. Therefore, thrombocytosis may be a cost-effective (ie, platelet count is an easy and inexpensive assay) and noninvasive biomarker for early cancer detection and poor prognosis.[113-117]

How platelets support tumor growth and metastasis

Novel insights into the molecular and cellular events of platelet-mediated cancer progression in the TME and blood are emerging hot topics.[9,11,120]

Platelets facilitate cancer to sustain proliferative signaling, resist cell death, and induce tumor angiogenesis Recent evidence suggests that platelets have a direct effect on cancer cell proliferation. Platelet transforming growth factor β (TGF-β) increased the proliferation of ovarian cancer cells.[121,122] Platelet microparticles also stimulated mitogen-activated protein kinases in lung carcinoma cells and increased cell proliferation.[123] Interestingly, patients with clear cell renal cell carcinoma have remarkably increased platelet isoform of phosphofructokinase (PFKP), a rate-controlling enzyme of the glycolytic pathway. Suppression of PFKP decreased glycolysis in clear cell renal cell carcinoma cells, impaired cell proliferation, and induced apoptosis[124,125]; it is unknown whether platelets could transfer their PFKP mRNA to cancer cells. In addition, platelets and platelet lysates could cause mitochondrial uncoupling and resistance to apoptosis in leukemia cells.[126] Collectively, these studies provide insights as to why patients with thrombocytosis usually have poor survival and enhanced resistance to chemotherapy. Indeed, experimental evidence shows that platelet depletion markedly reduced tumor weight and enhanced the efficacy of chemotherapy; conversely, platelet transfusion increased tumor size and decreased drug efficacy.[27,127] Thrombocytopenia-induced tumor hemorrhage may also improve drug delivery.[18] This raises a question whether we should increase the threshold for platelet transfusion in cancer patients with chemotherapy-induced thrombocytopenia.[27,128]

Platelets contain numerous proangiogenic factors, such as VEGF, platelet-derived growth factor (PDGF), basic fibroblast growth factor, and insulin-like growth factors.[15,104,129-132] These proangiogenic factors induce formation of tumor-infiltrating blood vessels and may promote proliferation/differentiation of cancer-associated pericytes and fibroblasts in the TME.[104,120] Platelets also contain antiangiogenic proteins, such as angiostatin, endostatin, thrombospondin-1, and PF4.[133,134] In the TME, cancer cells may use platelets to predominate the angiogenic environment, although the exact roles of these pro-/antiangiogenic factors and how platelets regulate their release remain to be determined. Evidence suggests that these different factors maybe compartmentalized into separate platelet granules,[57,58,104] or different granule proteins might be spatially packaged into distinct zones of the same granules,[135] allowing them to be preferentially released upon different stimuli.[136]

Therefore, tumor-associated platelets may prefer a proangiogenic phenotype. Indeed, clinical studies have demonstrated that platelets from cancer patients have increased levels of VEGF, PDGF, PF4, angiopoietin-1, matrix metalloproteinase-2, and IL-6.[105,120,137] The molecular mechanisms of phenotype changes remain largely unknown, but might be because tumor cells alter platelet transcriptome,[63,138] or platelets actively sequester tumor-derived angiogenic proteins,[139] which could then be delivered to the disseminated tumor sites.[123,140] Platelets also prevent intratumoral hemorrhage and stabilize the tumor vessels via secreting angiopoietin-1 and 5-HT.[141] Intriguingly, the antiangiogenic PF4 is significantly increased in platelets, but not in the plasma of tumor-bearing mice.[142] Whether platelet-associated PF4 could preferentially inhibit intratumoral hemorrhage through binding to heparan sulfate at injured/immature angiogenic sites remains to be established.[143] Altogether, these studies demonstrate that platelets facilitate

cancer to sustain proliferative signaling, resist cell death, and induce angiogenesis.

Platelets activate tumor invasion and support metastasis

Metastasis is still the biggest challenge in cancer care and the leading cause (~90%) of cancer-associated mortality.[11] Mounting evidence suggests that platelets play crucial roles in the "invasion-metastasis cascade."[11,144]

Platelets induce an invasive EMT phenotype of tumor cells and promote cell survival in blood circulation Cancer cell epithelial-mesenchymal transition (EMT) is considered a central mechanism by which transformed epithelial cells become more invasive.[62] Platelet-treated tumor cells have a downregulated E-cadherin level, loss of which is considered to be a fundamental event in EMT,[145] and an upregulated expression of mesenchymal markers, such as Snail, vimentin, fibronectin, and matrix metalloproteinase-9, and an increased prometastatic gene signature.[146] Thus, platelets can promote tumor cell migration and invasion into the surrounding microenvironment. Moreover, the activation of the tumor invasion-metastasis cascade by platelets depends on the synergistic activation of both platelet-derived TGF-β/Smad and NF-κB pathways in cancer cells, which are triggered by direct platelet–tumor cell contact.[146] Other platelet-released mediators have also been suggested to play a role in tumor EMT, such as prostaglandin (PG) E2, PDGF, and lysophosphatidic acid (LPA).[147-151]

The role of chemokine CCL5 in cancer invasion has been well recognized.[152] Mesenchymal stem cells within tumor stroma secrete CCL5 that induces a tumor-invasive behavior via CCR5 on cancer cells.[153] Anti-CCR5 therapy resulted in the repolarization of tumor-associated macrophages from protumor toward antitumor effects in patients with liver metastases.[154] Because platelet-secreted CCL5 can induce monocyte and T-lymphocyte adhesion/transmigration,[155,156] tumor-activated platelets may also release CCL5 to elicit tumor cell migration/invasion. In addition, a recent and elegant study identified a subpopulation of $CD36^+$ metastasis-initiating cells in tumors.[157] CD36 can drive metastasis by promoting fatty acid uptake and lipid metabolism.[157] Because platelets also express abundant CD36,[73] it is conceivable that platelets may transfer their CD36 to tumor cells and affect CD36-mediated metastasis. The observed antimetastatic effect of neutralizing anti-CD36 antibodies may result from their antiplatelet and/or tumor effects, including the potential inhibition of CD36-mediated platelet activation[74-76] and/or CD36-thrombospondin-1–mediated[21] TCIPA. These hypotheses remain to be examined.

After tumor cells detach from the primary site and intravasate into blood vessels, platelets are essential for tumor cell survival and transit in circulation.[9] Experimental metastasis is almost completely abolished in nuclear factor erythroid-derived 2 knockout mice that have impaired platelet production.[158] Platelets can rapidly associate with metastatic tumor cells via their receptors and cause TCIPA in circulation (see "TCIPA and formation of tumor-platelet aggregates"). Activated platelets can also provide procoagulant surfaces for cell-based thrombin generation,[19] which further activates platelets, leukocytes, and tumor cells, enhancing TCIPA. It was previously considered that platelets might passively provide a "shield" for the circulating tumor cells. However, we now know that TCIPA is not only important to protect circulating tumor cells against shear-induced cell membrane damage in circulation,[159] but is also an essential immune surveillance escape mechanism.[11,160]

Platelets facilitate tumor arrest at the endothelium, extravasation, and seeding The contribution of platelets to tumor arrest at the endothelium mainly involves adhesive interactions between platelets and endothelium, tumor cells, and leukocytes.[9,11] First, tumor cells, or tumor-activated platelets, can induce endothelial activation by their soluble factors, EVs, and proteases.[13,161] Activated endothelium can then directly recruit tumor cells, or platelet-tumor aggregates via several receptors, for instance, P-selectin, E-selectin, αVβ3 integrin, VWF, VCAM-1, and ICAM-1, and their ligands on tumor cells or platelets.[11,87] Platelets, likely via the platelet-derived cytokines such as CCL5,[56] engage monocytes to tumor cells and endothelium, which further enhances endothelial activation and indirectly facilitates tumor cell extravasation.[162,163] Furthermore, platelet-derived CXCL5 and CXCL7 chemokines recruit granulocytes and guide the formation of the early metastatic niche.[12] The formation of such cellular assemblies (ie, heteroaggregates of host–tumor cells) appears to be required for subsequent efficient metastasis.[11]

Moreover, available experimental evidence indicates that platelets can also directly enhance tumor extravasation.[9,11,146] As noted previously, platelet-released TGF-β and the direct platelet–tumor contact synergistically promoted cancer EMT and successful extravasation.[146] Additionally, platelet-derived LPA can support the progression of osteolytic bone metastases in breast cancer, likely involving the activation of the LPA receptor type 1 expressed on tumor cells.[164,165] Furthermore, tumor-activated platelets can generate autotaxin, an LPA-producing enzyme, which interacts with tumor αVβ3 integrin and thus generates more LPA to support metastasis.[150,151,166] It is currently unknown whether platelets could also support the adaptation of metastatic tumor cells to foreign tissue microenvironments and successful colonization, the last step of metastasis.[62] Clarifying the potential roles of platelets in enabling metastatic colonization represents an important agenda for future research.

Platelets promote tumor evasion of immune destruction

The immune system plays key roles in tumor immunosurveillance. Tumor-infiltrating lymphocytes (TILs) correlate with the improved survival of patients with melanoma, breast, esophageal, colorectal, and ovarian cancers.[167-170] However, surviving tumor cells turn to harness the immune system by hijacking its antitumor effects or attracting immunosuppressive cells.[171-175] The past 3 decades have seen the successful discovery of novel cancer immunotherapy, such as immune checkpoint inhibitors, chimeric antigen receptor T-cell therapy and vaccine treatments.[176]

Platelets have been recognized as immune cells.[6,55,56] However, their proinflammatory molecules, chemokines, and cytokines may facilitate not only inflammation and immune response but also TCIPA,[6,55,56] which relates to malignancy.[177] Interestingly, platelets may also be immune suppressive during tumorigenesis. It was previously found that platelets protected tumors from natural killer (NK) cell-mediated lysis in circulation[178,179] (and likely also in the TME). Tumor-activated platelets release a large amount of TGF-β, which downregulated the expression of NKG2D, the major receptor on NK cells to sense stress-associated molecules such as major histocompatibility complex class I chain-related

proteins A and B,[180,181] impairing interferon-γ production and NK cell cytotoxicity.[182] TGF-β also suppressed mTOR activity in NK cells, which inhibited NK cell activation/function.[183] Additionally, platelets can transfer their major histocompatibility complex class I molecules to tumor cells,[184] and tumor cells can also resemble platelets by displaying several platelet receptor markers.[185] This "platelet mimicry" allows tumors to evade attacks from NK cells.[184,185] Another mechanism may involve platelet glucocorticoid-induced tumor necrosis factor receptor ligand-mediated interference with NK cell immunosurveillance.[186] Overall, these studies demonstrate that platelets protect tumor cells from NK cell–mediated lysis.

Tumor-associated platelets may also affect the activity of other immune cells through multiple receptors and a range of immunomodulatory chemokines, for instance, leukocyte trafficking.[187,188] Recent evidence shows that metastatic breast cancer cells induce neutrophils to form metastasis-supporting neutrophil extracellular traps.[189,190] It is possible that tumor-associated platelets may mediate neutrophil trafficking and extravasation,[191] for example, via interactions between platelet GPIbα and neutrophil αMβ2 integrin,[192] and may form tumor–platelet–neutrophil complexes to potentially enhance immune escape.

In addition, platelets may "paralyze" TIL by secreting large amounts of TGF-β, or by TGF-β delivered from platelet EV. Importantly, recent studies have provided some insights; glycoprotein A repetitions predominant (GARP), which is the cell surface docking receptor for latent TGF-β that causes TGF-β activation, was overexpressed in patients with breast, lung, and colon cancers. The TGF-β-GARP axis in the TME promotes T regulatory cell-mediated immune suppression.[193] Most recently, experimental evidence showed that the majority of functional TGF-β is actually generated by platelets, both systemically and locally at the site of tumor, because platelets also express GARP receptor, rather than secrete TGF-β alone.[194] Furthermore, a combination of antiplatelet agents (eg, aspirin and clopidogrel) markedly improves the efficacy of adoptive T-cell therapy against cancer in animal models.[194] These data indicate that platelets are able to directly subvert T-cell immunity. Interestingly, evidence emerged suggesting platelets and platelet-derived microparticles can also infiltrate tumors[118,195]; it is currently unclear whether they can directly interact with TIL. These questions remain to be addressed.

Based on the intensive tumor–platelet interactions, other applications have also been suggested. For example, conjugation of platelets to the anti-programmed death-ligand 1 antibody facilitates the delivery of anti-programmed death-ligand 1 to the site of postsurgical residual microtumors and circulating tumor cells, thus reducing postsurgical tumor recurrence and experimental metastasis.[196] Altogether, platelets may have "carcinogenic" potential and thrombocytosis may facilitate malignancy, which might therefore be a desirable target for cancer therapy.

Aspirin protects against cancer

Since its first synthesis in 1897, aspirin, a nonsteroidal anti-inflammatory drug, has been one of the most widely used medications to reduce pain, fever, inflammation, and platelet activity. Mounting evidence has supported its new use in cancer prevention, reducing metastasis and mortality, especially for colorectal cancer (CRC).[3,197-204] In 2016, the US Preventive Services Task Force incorporated the prophylactic effect of low-dose aspirin on CRC and recommends the initiation of daily low-dose aspirin (eg, 75-100 mg/day) for at least 10 years in adults aged 50 to 69 years with specific CVD risk.[5] In contrast, considering the risks (eg, bleeding tendency, gastrointestinal disorders) vs unproven benefits of long-term aspirin use, the European Guidelines in clinical practice do not support its role in primary prevention.[205,206] However, some investigators believe that low-dose aspirin does not significantly cause bleeding complications in average-risk individuals.[206-209]

Other evidence suggests that aspirin might also reduce the incidence of nongastrointestinal cancers, such as cholangiocarcinoma, breast, prostate, lung, endometrial, pancreatic, and ovarian cancers.[210-217] However, these findings should be interpreted with caution because of study heterogeneity, and the clinical data are not always consistently promising.[197,202,211] Large randomized controlled trials to further determine its contribution are warranted.

Platelet-mediated mechanism of aspirin

Aspirin is an irreversible cyclooxygenase (COX) inhibitor through acetylation of a serine residue that reduces the synthesis of prostanoids, such as PGE2 and TXA2, from arachidonic acid. COX-1 is constitutively expressed in platelets and gastric epithelial cells and is responsible for the generation of TXA2 in platelets and the basal production of cytoprotective prostaglandins in the gastric mucosa. COX-2 is not normally expressed in most cells (except some tissues such as endothelium); however, it is progressively overexpressed in many cancer cells, including colorectal, breast, gastric, lung, and pancreatic cancers and melanoma. A critical event in tumorigenesis and metastasis involves the enhanced synthesis of PGE2 by COX, which in turn enhances tumor proliferation, angiogenesis, differentiation, inflammation, and immune escape.[218-220] The capacity of aspirin to inhibit COX activity and prostanoid production has been widely considered the central mechanism of its anticancer effects; however, a significant gap occurs when determining how much of the effect is platelet-dependent and how much is owed to the direct inhibition of COX in other cells, such as tumor cells.

It was considered that COX-2 inhibition by aspirin is essential for its anticancer action. Aspirin use was found to correlate with reduced risk of CRC in patients that overexpressed COX-2, but not in those who had weak or absent COX-2 expression.[221] However, subsequent clinical and pharmacology studies suggest that the antiplatelet effect of aspirin (ie, permanent inactivation of platelet COX-1) is sufficient and necessary for its anticancer action.[3,198-200,202] First, similar effects of aspirin at doses of 75 to 300 mg/day have been shown to reduce cancer incidence, metastasis, and mortality, with 75 mg/day being as effective as higher doses; and dosing at 24-hour intervals appears to be sufficient.[198-200,202] Moreover, chemopreventive effects were found with a low-dose, slow-release formulation of aspirin that was designed to specifically inhibit platelet function with few systemic effects.[200] Also, aspirin has a short half-life (20 minutes) in human circulation. Daily low-dose aspirin is able to irreversibly and completely inhibit platelet COX-1 activity and TXA2 production; consequently, the profound

inhibition of platelet function (eg, TCIPA) by aspirin persists throughout the dose interval (ie, 24 hours). In contrast, this dose cannot achieve sustained inhibition of COX-2 in nucleated cells because nucleated cells have the capacity to de novo synthesize COX isozymes within a few hours, whereas platelets cannot.[222] Higher doses of aspirin (eg, 650 mg 3 times/day) have been shown to be required for sustained inhibition of COX-2.[202,223]

Experimental evidence with aspirin also demonstrates a platelet-related mechanism. Aspirin inhibited platelet-induced angiogenesis after exposure to breast cancer cells,[104] reduced platelet-promoted colon and pancreatic cancer cell proliferation,[224] and rescued platelet-accelerated metastatic potential of colon cancer cells, which is likely through inhibiting platelet COX-1–mediated TXA2 and PGE2 biosynthesis.[149] Moreover, aspirin plus clopidogrel improved the efficacy of adoptive T-cell therapy against cancer[194] and prevented hepatitis B virus–associated liver cancer in animal models.[225] Combined, these findings underscore a platelet-dependent effect of low-dose aspirin in cancer prevention.[226,227] Because platelets play important roles in tumorigenesis and metastasis, platelet inhibition may bring greater benefits than once thought.

Intriguingly, the blockade of platelet COX-1 activity may also negatively regulate the expression and function of COX-2 in adjacent cancer cells.[3,202,228] Studies showed that platelets induced overexpression of COX-2 in colon carcinoma cells through direct platelet–cancer cell interaction and release of paracrine lipid and protein mediators.[148,208] Platelet-derived Wnt caused β-catenin translocation into the nucleus and the rapid increase of COX-2 mRNA in cancer cells.[227] Aspirin could inhibit platelet activation and platelet-mediated COX-2 expression in adjacent nucleated cells at sites of mucosal injury.[229,230] However, deciphering the crosstalk between COX-1 and COX-2 in different cells during cancer development is a challenging question to be further investigated.

COX-independent mechanisms of aspirin in cancer have also been suggested, including modifications of NF-κB and RUNX1,[231] induction of cancer cell apoptosis, reversal of hypermethylation of tumor suppressor genes, downregulation of mutation-inducing DNA damage, and acetylation of intracellular RNA.[232-234] However, most of these effects have been characterized in vitro using supratherapeutic concentrations of aspirin, and the in vivo effects and evidence by low-dose aspirin is lacking.

Opportunities and challenges for other antiplatelet agents

As mentioned for antiplatelet agents,[99] aspirin and several COX-1 inhibitors have been used in patients for decades to centuries. Integrin αIIbβ3 antagonists and adenosine 5′-diphosphate receptor (P2Y12) antagonist clopidogrel were prescribed to patients in the 1990s. Several newer P2Y12 antagonists and a thrombin receptor (PAR1) antagonist[235] have also been recently approved by US Food and Drug Administration. Although aside from aspirin, little clinical information is available regarding other antiplatelet agents in cancer[81]; it is predictable that some of them may be beneficial for patients.[79-81,194,225,235,236]

Several other antiplatelet drugs are in the preclinical stage or different phases of clinical trials, and some may be available in the market in the near future.[237] Besides P-selectin inhibitors, GPVI and GPIbα antagonists are under development.[99] Although GPVI was considered as the platelet activation receptor for fibrillar collagen on subendothelial matrix, a recent study demonstrated that it bound to fibrin(ogen),[238,239] leading to the possible involvement of GPVI in TCIPA, which may explain its supportive role in metastasis in mice.[240] It will be interesting to reexamine whether GPVI antagonists have confounding effects on tumor metastasis.[241] GPIbα antagonists should be highlighted because anti-GPIbα may have dual roles in metastasis.[91,94] As reported by us and others, antibodies against GPIbα inhibited not only VWF binding, but also its interactions with thrombin[95] and other molecules such as αMβ2 integrin[192] and P-selectin,[242] which may have broad inhibitory effects on TCIPA and tumor–platelet–leukocyte heterotypical aggregation. Additionally, as we recently observed, anti-GPIbα antibodies can decrease thrombopoietin generation,[119] which may inhibit tumor-induced thrombocytosis. Other emerging antiplatelet agents include those targeting platelet-activating receptors (eg, anti-CD36,[157] anti-CLEC 2,[71,72] EP3 receptor antagonists[149,243]) and inhibitory receptors (eg, anti-PECAM-1, CEACAM1),[70,244] etc. Furthermore, other agents of platelet blockade, such as anticoagulants that inhibit thrombin-induced platelet activation/TCIPA, non-aspirin nonsteroidal anti-inflammatory drugs and plant-based food products (eg, anthocyanins) may also have antitumor effects.[245-247] Characterizing these new agents will certainly advance our knowledge and treatment to control cancer (Table 1).

Although we focus here on "how cancer changes platelets to be more cancer-friendly," one cannot exclude the potential "dual" (ie, supportive and inhibitive) roles of platelets in tumor progression. An elegant study recently showed that platelet microparticles can infiltrate solid tumors and deliver miR-24, which induces tumor cell apoptosis and suppresses tumor growth.[190,195] In fact, platelets are versatile and part of the innate immune system. They can modify adaptive immunity and therefore may significantly contribute to immunosurveillance.[53,55,56] Furthermore, although the prevailing view is that platelets are proinflammatory and immune supportive, our recent data unveiled their immune-suppressive activities following platelet desialylation.[248] Also, platelets contain both pro- and anti-angiogenic factors. We therefore cannot exclude that different antiplatelet drugs, the same drug in different doses or patients may have different consequences or even detrimental effects. A better understanding of the pro- and antitumor activities of platelets could be the next big breakthrough that will advance our knowledge in platelet–cancer interactions for therapeutic benefits. More basic and clinical studies should be able to address these questions.

Summary

This article highlights evidence for intimate crosstalk between cancer and platelets (Figure 1). Tumor-associated platelet proteins, RNA profiles, and thrombocytosis may be useful biomarkers for cancer screening, diagnosis, prognosis, and treatment monitoring. Further clinical trials are needed to identify and validate cancers that are closely linked with these signatures. In addition, TEP-based liquid biopsy assay is emerging, although further characterization is required before it

can be a reliable diagnostic tool. Reciprocally, platelets can further support tumorigenesis and metastasis. Targeting platelet–cancer crosstalk may represent a novel and promising antitumor strategy. Several prospective clinical trials are currently evaluating the benefits of adjuvant aspirin treatment in patients with colorectal, breast, esophageal, ovarian, or lung cancers. Notably, however, the role of chronic platelet inhibition in cancers is not always consistent. Dual antiplatelet therapy by prasugrel, ticagrelor, or vorapaxar on top of aspirin was shown to correlate with excess tumor growth and cancer-associated death in several clinical trials.[249-252] The exact mechanisms are still unclear, but it might be due to the impairment of the possible antitumor activity of platelets.[195,253]

The dynamic requisites of tumor cells during tumorigenesis and metastasis have given rise to challenging questions to fully understand the exact roles of platelets at different stages of cancer; how platelets may balance their pro- and antitumor activities, which might involve distinct signaling pathways and molecule variants; and why platelet inhibition by aspirin works best in certain cancers. Furthermore, to identify individuals for whom the benefits outweigh the hazards (eg, hemorrhage, thrombocytopenia, gastrointestinal disorders, immune alterations) and determine sensitive tumor types for antiplatelet treatment are of great importance for personalized medicine. Other challenges such as the requirement for intravenous infusion of some antiplatelet agents (eg, αIIbβ3 antagonists) may add difficulties to the clinical trials. Nonetheless, adjuvant treatment with aspirin and other antiplatelet agents may open a new era and opportunity for antitumor therapy.

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Footnotes

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REFERENCES

1. Harding MC, Sloan CD, Merrill RM, Harding TM, Thacker BJ, Thacker EL. Transition from cardiovascular disease to cancer as the leading cause of death in US states, 1999-2013 [abstract]. *Circulation*. 2016; 133(suppl 1):AMP67. Abstract MP67.
2. Weir HK, Anderson RN, Coleman King SM, et al. Heart disease and cancer deaths - trends and projections in the United States, 1969-2020. *Prev Chronic Dis*. 2016;13:E157.
3. Drew DA, Cao Y, Chan AT. Aspirin and colorectal cancer: the promise of precision chemoprevention. *Nat Rev Cancer*. 2016; 16(3):173-186.
4. Chia WK, Ali R, Toh HC. Aspirin as adjuvant therapy for colorectal cancer–reinterpreting paradigms. *Nat Rev Clin Oncol*. 2012;9(10): 561-570.
5. Bibbins-Domingo K, U.S. Preventive Services Task Force. Aspirin use for the primary prevention of cardiovascular disease and colorectal cancer: U.S. Preventive Services Task Force Recommendation Statement. *Ann Intern Med*. 2016;164(12):836-845.
6. Xu XR, Zhang D, Oswald BE, et al. Platelets are versatile cells: new discoveries in hemostasis, thrombosis, immune responses, tumor metastasis and beyond. *Crit Rev Clin Lab Sci*. 2016;53(6):409-430.
7. Lefrançais E, Ortiz-Muñoz G, Caudrillier A, et al. The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. *Nature*. 2017;544(7648):105-109.
8. Davis RB, Theologides A, Kennedy BJ. Comparative studies of blood coagulation and platelet aggregation in patients with cancer and nonmalignant diseases. *Ann Intern Med*. 1969;71(1):67-80.
9. Gay LJ, Felding-Habermann B. Contribution of platelets to tumour metastasis. *Nat Rev Cancer*. 2011;11(2):123-134.
10. Meikle CK, Kelly CA, Garg P, Wuescher LM, Ali RA, Worth RG. Cancer and Thrombosis: The Platelet Perspective. *Front Cell Dev Biol*. 2017;4:147.
11. Labelle M, Hynes RO. The initial hours of metastasis: the importance of cooperative host-tumor cell interactions during hematogenous dissemination. *Cancer Discov*. 2012;2(12):1091-1099.
12. Labelle M, Begum S, Hynes RO. Platelets guide the formation of early metastatic niches. *Proc Natl Acad Sci USA*. 2014; 111(30):E3053-E3061.
13. Franco AT, Corken A, Ware J. Platelets at the interface of thrombosis, inflammation, and cancer. *Blood*. 2015;126(5):582-588.
14. Leblanc R, Peyruchaud O. Metastasis: new functional implications of platelets and megakaryocytes. *Blood*. 2016;128(1): 24-31.
15. Li N. Platelets in cancer metastasis: to help the "villain" to do evil. *Int J Cancer*. 2016; 138(9):2078-2087.
16. Ruggeri ZM. Platelets in atherothrombosis. *Nat Med*. 2002;8(11):1227-1234.
17. Wang Y, Andrews M, Yang Y, et al. Platelets in thrombosis and hemostasis: old topic with new mechanisms. *Cardiovasc Hematol Disord Drug Targets*. 2012;12(2):126-132.
18. Demers M, Wagner DD. Targeting platelet function to improve drug delivery. *OncoImmunology*. 2012;1(1):100-102.
19. Roberts HR, Hoffman M, Monroe DM. A cell-based model of thrombin generation. *Semin Thromb Hemost*. 2006;32(S 1 Suppl 1): 32-38.
20. Gremmel T, Frelinger AL III, Michelson AD. Platelet physiology. *Semin Thromb Hemost*. 2016;42(3):191-204.
21. Wang Y, Gallant RC, Ni H. Extracellular matrix proteins in the regulation of thrombus formation. *Curr Opin Hematol*. 2016;23(3): 280-287.
22. Gui T, Reheman A, Funkhouser WK, et al. In vivo response to vascular injury in the absence of factor IX: examination in factor IX knockout mice. *Thromb Res*. 2007;121(2): 225-234.
23. Zdravic D, Yougbare I, Vadasz B, et al. Fetal and neonatal alloimmune thrombocytopenia. *Semin Fetal Neonatal Med*. 2016;21(1):19-27.
24. Kjeldsen-Kragh J, Ni H, Skogen B. Towards a prophylactic treatment of HPA-related foetal and neonatal alloimmune thrombocytopenia. *Curr Opin Hematol*. 2012;19(6):469-474.
25. Zeng Q, Zhu L, Tao L, et al. Relative efficacy of steroid therapy in immune thrombocytopenia mediated by anti-platelet GPIIbIIIa

versus GPIbα antibodies. *Am J Hematol*. 2012;87(2):206-208.

26. Tao L, Zeng Q, Li J, et al. Platelet desialylation correlates with efficacy of first-line therapies for immune thrombocytopenia. *J Hematol Oncol*. 2017;10(1):46.

27. Demers M, Ho-Tin-Noé B, Schatzberg D, Yang JJ, Wagner DD. Increased efficacy of breast cancer chemotherapy in thrombocytopenic mice. *Cancer Res*. 2011;71(5):1540-1549.

28. Rodeghiero F, Michel M, Gernsheimer T, et al. Standardization of bleeding assessment in immune thrombocytopenia: report from the International Working Group. *Blood*. 2013;121(14):2596-2606.

29. Xu XR, Gallant RC, Ni H. Platelets, immune-mediated thrombocytopenias, and fetal hemorrhage. *Thromb Res*. 2016;141(Suppl 2):S76-S79.

30. Yougbaré I, Lang S, Yang H, et al. Maternal anti-platelet β3 integrins impair angiogenesis and cause intracranial hemorrhage. *J Clin Invest*. 2015;125(4):1545-1556.

31. Mackman N. Triggers, targets and treatments for thrombosis. *Nature*. 2008;451(7181):914-918.

32. Zhu G, Zhang Q, Reddy EC, et al. The integrin PSI domain has an endogenous thiol isomerase function and is a novel target for antiplatelet therapy. *Blood*. 2017;129(13):1840-1854.

33. Reheman A, Xu X, Reddy EC, Ni H. Targeting activated platelets and fibrinolysis: hitting two birds with one stone. *Circ Res*. 2014;114(7):1070-1073.

34. Schulman S. How I treat recurrent venous thromboembolism in patients receiving anticoagulant therapy. *Blood*. 2017;129(25):3285-3293.

35. Tesselaar ME, Romijn FP, Van Der Linden IK, Prins FA, Bertina RM, Osanto S. Microparticle-associated tissue factor activity: a link between cancer and thrombosis? *J Thromb Haemost*. 2007;5(3):520-527.

36. 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.

37. Tilley RE, Holscher T, Belani R, Nieva J, Mackman N. Tissue factor activity is increased in a combined platelet and microparticle sample from cancer patients. *Thromb Res*. 2008;122(5):604-609.

38. Geddings JE, Mackman N. Tumor-derived tissue factor-positive microparticles and venous thrombosis in cancer patients. *Blood*. 2013;122(11):1873-1880.

39. Ni H, Yuen PS, Papalia JM, et al. Plasma fibronectin promotes thrombus growth and stability in injured arterioles. *Proc Natl Acad Sci USA*. 2003;100(5):2415-2419.

40. Reheman A, Yang H, Zhu G, et al. Plasma fibronectin depletion enhances platelet aggregation and thrombus formation in mice lacking fibrinogen and von Willebrand factor. *Blood*. 2009;113(8):1809-1817.

41. Reheman A, Gross P, Yang H, et al. Vitronectin stabilizes thrombi and vessel occlusion but plays a dual role in platelet aggregation. *J Thromb Haemost*. 2005;3(5):875-883.

42. Ni H, Denis CV, Subbarao S, et al. Persistence of platelet thrombus formation in arterioles of mice lacking both von Willebrand factor and fibrinogen. *J Clin Invest*. 2000;106(3):385-392.

43. Ni H, Papalia JM, Degen JL, Wagner DD. Control of thrombus embolization and fibronectin internalization by integrin alpha IIb beta 3 engagement of the fibrinogen gamma chain. *Blood*. 2003;102(10):3609-3614.

44. Yang H, Reheman A, Chen P, et al. Fibrinogen and von Willebrand factor-independent platelet aggregation in vitro and in vivo. *J Thromb Haemost*. 2006;4(10):2230-2237.

45. Jirousková M, Chereshnev I, Väänänen H, Degen JL, Coller BS. Antibody blockade or mutation of the fibrinogen gamma-chain C-terminus is more effective in inhibiting murine arterial thrombus formation than complete absence of fibrinogen. *Blood*. 2004;103(6):1995-2002.

46. Zhai Z, Wu J, Xu X, et al. Fibrinogen controls human platelet fibronectin internalization and cell-surface retention. *J Thromb Haemost*. 2007;5(8):1740-1746.

47. Xu X, Wu J, Zhai Z, et al. A novel fibrinogen Bbeta chain frameshift mutation in a patient with severe congenital hypofibrinogenaemia. *Thromb Haemost*. 2006;95(6):931-935.

48. Hou Y, Carrim N, Wang Y, Gallant RC, Marshall A, Ni H. Platelets in hemostasis and thrombosis: Novel mechanisms of fibrinogen-independent platelet aggregation and fibronectin-mediated protein wave of hemostasis [published online ahead of print 30 October 2015]. *J Biomed Res*. doi: 10.7555/JBR.29.20150121.

49. Ni H, Freedman J. Platelets in hemostasis and thrombosis: role of integrins and their ligands. *Transfus Apheresis Sci*. 2003;28(3):257-264.

50. Wang Y, Reheman A, Spring CM, et al. Plasma fibronectin supports hemostasis and regulates thrombosis. *J Clin Invest*. 2014;124(10):4281-4293.

51. Wang Y, Ni H. Fibronectin: extra domain brings extra risk? *Blood*. 2015;125(20):3043-3044.

52. Dunne E, Spring CM, Reheman A, et al. Cadherin 6 has a functional role in platelet aggregation and thrombus formation. *Arterioscler Thromb Vasc Biol*. 2012;32(7):1724-1731.

53. Lindemann S, Krämer B, Seizer P, Gawaz M. Platelets, inflammation and atherosclerosis. *J Thromb Haemost*. 2007;5(Suppl 1):203-211.

54. Murphy AJ, Bijl N, Yvan-Charvet L, et al. Cholesterol efflux in megakaryocyte progenitors suppresses platelet production and thrombocytosis. *Nat Med*. 2013;19(5):586-594.

55. Li C, Li J, Li Y, et al. Crosstalk between platelets and the immune system: old systems with new discoveries. *Adv Hematol*. 2012;2012:384685.

56. Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. *Nat Rev Immunol*. 2011;11(4):264-274.

57. Italiano JE Jr, Richardson JL, Patel-Hett S, et al. Angiogenesis is regulated by a novel mechanism: pro- and antiangiogenic proteins are organized into separate platelet alpha granules and differentially released. *Blood*. 2008;111(3):1227-1233.

58. Chatterjee M, Huang Z, Zhang W, et al. Distinct platelet packaging, release, and surface expression of proangiogenic and antiangiogenic factors on different platelet stimuli. *Blood*. 2011;117(14):3907-3911.

59. Hess PR, Rawnsley DR, Jakus Z, et al. Platelets mediate lymphovenous hemostasis to maintain blood-lymphatic separation throughout life. *J Clin Invest*. 2014;124(1):273-284.

60. Herzog BH, Fu J, Wilson SJ, et al. Podoplanin maintains high endothelial venule integrity by interacting with platelet CLEC-2. *Nature*. 2013;502(7469):105-109.

61. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000;100(1):57-70.

62. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-674.

63. Best MG, Sol N, Kooi I, et al. RNA-Seq of tumor-educated platelets enables blood-based pan-cancer, multiclass, and molecular pathway cancer diagnostics. *Cancer Cell*. 2015;28(5):666-676.

64. Joosse SA, Pantel K. Tumor-educated platelets as liquid biopsy in cancer patients. *Cancer Cell*. 2015;28(5):552-554.

65. Best MG, Sol N. In 't Veld SGJG, et al. Swarm intelligence-enhanced detection of non-small-cell lung cancer using tumor-educated platelets. *Cancer Cell*. 2017;32(2):238-252.

66. Chi KR. The tumour trail left in blood. *Nature*. 2016;532(7598):269-271.

67. Trousseau A. Phlegmasia Alba Dolens. Clinique medicale de l'Hotel-Dieu de Paris. London: New Syndeham Society; 1865:94-96.

68. Bastida E, Ordinas A. Platelet contribution to the formation of metastatic foci: the role of cancer cell-induced platelet activation. *Haemostasis*. 1988;18(1):29-36.

69. Clemetson KJ, Clemetson JM. Platelet receptors. In: Michelson AD, ed. Platelets. London: Academic Press; 2013:169-194.

70. Wong C, Liu Y, Yip J, et al. CEACAM1 negatively regulates platelet-collagen interactions and thrombus growth in vitro and in vivo. *Blood*. 2009;113(8):1818-1828.

71. Shirai T, Inoue O, Tamura S, et al. C-type lectin-like receptor 2 promotes hematogenous tumor metastasis and prothrombotic state in tumor-bearing mice. *J Thromb Haemost*. 2017;15(3):513-525.

72. Astarita JL, Acton SE, Turley SJ. Podoplanin: emerging functions in development, the immune system, and cancer. *Front Immunol*. 2012;3:283.

73. Wu G, Zhou Y, Li L, et al. Platelet immunology in China: research and clinical applications. *Transfus Med Rev*. 2017;31(2):118-125.

74. Podrez EA, Byzova TV, Febbraio M, et al. Platelet CD36 links hyperlipidemia, oxidant stress and a prothrombotic phenotype. *Nat Med*. 2007;13(9):1086-1095.

75. Ni H. The platelet "sugar high" in diabetes. *Blood*. 2012;119(25):5949-5951.

76. Zhu W, Li W, Silverstein RL. Advanced glycation end products induce a prothrombotic phenotype in mice via interaction with platelet CD36. *Blood*. 2012;119(25):6136-6144.

77. Cameron-Vendrig A, Reheman A, Siraj MA, et al. Glucagon-like peptide 1 receptor activation attenuates platelet aggregation and thrombosis. *Diabetes*. 2016;65(6):1714-1723.

78. Patel S, Huang YW, Reheman A, et al. The cell motility modulator Slit2 is a potent inhibitor of platelet function. *Circulation*. 2012;126(11):1385-1395.

79. Felding-Habermann B, O'Toole TE, Smith JW, et al. Integrin activation controls metastasis in human breast cancer. *Proc Natl Acad Sci USA*. 2001;98(4):1853-1858.

80. Desgrosellier JS, Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities [published correction appears in *Nat Rev Cancer*. 2010;10:890]. *Nat Rev Cancer*. 2010;10(1):9-22.

81. Bakewell SJ, Nestor P, Prasad S, et al. Platelet and osteoclast beta3 integrins are critical for bone metastasis. *Proc Natl Acad Sci USA*. 2003;100(24):14205-14210.

82. Mammadova-Bach E, Zigrino P, Brucker C, et al. Platelet integrinα6β1 controls lung metastasis through direct binding to cancer cell-derived ADAM9. *JCI Insight*. 2016;1(14):e88245.

83. Chen C, He Z, Sai P, et al. Inhibition of human CD24 binding to platelet-bound P-selectin by monoclonal antibody. *Proc West Pharmacol Soc*. 2004;47:28-29.

84. Stone JP, Wagner DD. P-selectin mediates adhesion of platelets to neuroblastoma and small cell lung cancer. *J Clin Invest*. 1993;92(2):804-813.

85. Garcia J, Callewaert N, Borsig L. P-selectin mediates metastatic progression through binding to sulfatides on tumor cells. *Glycobiology*. 2007;17(2):185-196.

86. Kim YJ, Borsig L, Varki NM, Varki A. P-selectin deficiency attenuates tumor growth and metastasis. *Proc Natl Acad Sci USA*. 1998;95(16):9325-9330.

87. Kim YJ, Borsig L, Han HL, Varki NM, Varki A. Distinct selectin ligands on colon carcinoma mucins can mediate pathological interactions among platelets, leukocytes, and endothelium. *Am J Pathol*. 1999;155(2):461-472.

88. Yang H, Lang S, Zhai Z, et al. Fibrinogen is required for maintenance of platelet intracellular and cell-surface P-selectin expression. *Blood*. 2009;114(2):425-436.

89. Yu LX, Yan L, Yang W, et al. Platelets promote tumour metastasis via interaction between TLR4 and tumour cell-released high-mobility group box1 protein. *Nat Commun*. 2014;5:5256.

90. Riedl J, Preusser M, Nazari PM, et al. Podoplanin expression in primary brain tumors induces platelet aggregation and increases risk of venous thromboembolism. *Blood*. 2017;129(13):1831-1839.

91. Jain S, Zuka M, Liu J, et al. Platelet glycoprotein Ib alpha supports experimental lung metastasis. *Proc Natl Acad Sci USA*. 2007;104(21):9024-9028.

92. Mojiri A, Stoletov K, Carrillo MA, et al. Functional assessment of von Willebrand factor expression by cancer cells of non-endothelial origin. *Oncotarget*. 2017;8(8):13015-13029.

93. Lei X, Reheman A, Hou Y, et al. Anfibatide, a novel GPIb complex antagonist, inhibits platelet adhesion and thrombus formation in vitro and in vivo in murine models of thrombosis. *Thromb Haemost*. 2014;111(2):279-289.

94. Erpenbeck L, Nieswandt B, Schön M, Pozgajova M, Schön MP. Inhibition of platelet GPIb alpha and promotion of melanoma metastasis. *J Invest Dermatol*. 2010;130(2):576-586.

95. Li C, Piran S, Chen P, et al. The maternal immune response to fetal platelet GPIbα causes frequent miscarriage in mice that can be prevented by intravenous IgG and anti-FcRn therapies. *J Clin Invest*. 2011;121(11):4537-4547.

96. Li J, van der Wal DE, Zhu G, et al. Desialylation is a mechanism of Fc-independent platelet clearance and a therapeutic target in immune thrombocytopenia. *Nat Commun*. 2015;6(1):7737.

97. Bergmeier W, Rackebrandt K, Schröder W, Zirngibl H, Nieswandt B. Structural and functional characterization of the mouse von Willebrand factor receptor GPIb-IX with novel monoclonal antibodies. *Blood*. 2000;95(3):886-893.

98. Quach ME, Dragovich MA, Chen W, et al. Fc-independent immune thrombocytopenia via mechanomolecular signaling in platelets. *Blood*. 2018;131(7):787-796.

99. Xu XR, Carrim N, Neves MA, et al. Platelets and platelet adhesion molecules: novel mechanisms of thrombosis and anti-thrombotic therapies. *Thromb J*. 2016;14(S1 Suppl 1):29.

100. Provost P. The clinical significance of platelet microparticle-associated microRNAs. *Clin Chem Lab Med*. 2017;55(5):657-666.

101. Kim HK, Song KS, Park YS, et al. Elevated levels of circulating platelet microparticles, VEGF, IL-6 and RANTES in patients with gastric cancer: possible role of a metastasis predictor. *Eur J Cancer*. 2003;39(2):184-191.

102. Helley D, Banu E, Bouziane A, et al. Platelet microparticles: a potential predictive factor of survival in hormone-refractory prostate cancer patients treated with docetaxel-based chemotherapy. *Eur Urol*. 2009;56(3):479-484.

103. Liang H, Yan X, Pan Y, et al. MicroRNA-223 delivered by platelet-derived microvesicles promotes lung cancer cell invasion via targeting tumor suppressor EPB41L3. *Mol Cancer*. 2015;14(1):58.

104. Battinelli EM, Markens BA, Italiano JE Jr. Release of angiogenesis regulatory proteins from platelet alpha granules: modulation of physiologic and pathologic angiogenesis. *Blood*. 2011;118(5):1359-1369.

105. Holmes CE, Levis JE, Schneider DJ, et al. Platelet phenotype changes associated with breast cancer and its treatment. *Platelets*. 2016;27(7):703-711.

106. Calverley DC, Phang TL, Choudhury QG, et al. Significant downregulation of platelet gene expression in metastatic lung cancer. *Clin Transl Sci*. 2010;3(5):227-232.

107. Nilsson RJ, Balaj L, Hulleman E, et al. Blood platelets contain tumor-derived RNA biomarkers. *Blood*. 2011;118(13):3680-3683.

108. Nilsson RJ, Karachaliou N, Berenguer J, et al. Rearranged EML4-ALK fusion transcripts sequester in circulating blood platelets and enable blood-based crizotinib response monitoring in non-small-cell lung cancer. *Oncotarget*. 2016;7(1):1066-1075.

109. Meehan K, Vella LJ. The contribution of tumour-derived exosomes to the hallmarks of cancer. *Crit Rev Clin Lab Sci*. 2016;53(2):121-131.

110. Fendler A, Stephan C, Yousef GM, Kristiansen G, Jung K. The translational potential of microRNAs as biofluid markers of urological tumours. *Nat Rev Urol*. 2016;13(12):734-752.

111. Skog J, Würdinger T, van Rijn S, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol*. 2008;10(12):1470-1476.

112. Di Meo A, Bartlett J, Cheng Y, Pasic MD, Yousef GM. Liquid biopsy: a step forward towards precision medicine in urologic malignancies. *Mol Cancer*. 2017;16(1):80.

113. Long Y, Wang T, Gao Q, Zhou C. Prognostic significance of pretreatment elevated platelet count in patients with colorectal cancer: a meta-analysis. *Oncotarget*. 2016;7(49):81849-81861.

114. Gao L, Zhang H, Zhang B, Zhang L, Wang C. Prognostic value of combination of preoperative platelet count and mean platelet volume in patients with resectable non-small cell lung cancer. *Oncotarget*. 2017;8(9):15632-15641.

115. Bailey SE, Ukoumunne OC, Shephard E, Hamilton W. How useful is thrombocytosis in predicting an underlying cancer in primary care? a systematic review. *Fam Pract*. 2017;34(1):4-10.

116. Stone RL, Nick AM, McNeish IA, et al. Paraneoplastic thrombocytosis in ovarian cancer [published correction appears in *N Engl J Med*. 2012;367(18):1768]. *N Engl J Med*. 2012;366(7):610-618.

117. Lin RJ, Afshar-Kharghan V, Schafer AI. Paraneoplastic thrombocytosis: the secrets of tumor self-promotion. *Blood*. 2014;124(2):184-187.

118. Pucci F, Rickelt S, Newton AP, et al. PF4 promotes platelet production and lung cancer growth. *Cell Reports*. 2016;17(7):1764-1772.

119. Xu M, Ma L, Carrim N, et al. Platelet GPIbalpha is important for thrombopoietin production and thrombopoietin-induced platelet generation. *Blood*. 2015;126(23):12.

120. Yan M, Jurasz P. The role of platelets in the tumor microenvironment: from solid tumors to leukemia. *Biochim Biophys Acta*. 2016;1863(3):392-400.

121. Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor beta in human disease. *N Engl J Med*. 2000;342(18):1350-1358.

122. Cho MS, Bottsford-Miller J, Vasquez HG, et al. Platelets increase the proliferation of ovarian cancer cells. *Blood*. 2012;120(24):4869-4872.

123. Janowska-Wieczorek A, Wysoczynski M, Kijowski J, et al. Microvesicles derived from activated platelets induce metastasis and angiogenesis in lung cancer. *Int J Cancer*. 2005;113(5):752-760.

124. Wang J, Zhang P, Zhong J, et al. The platelet isoform of phosphofructokinase contributes to metabolic reprogramming and maintains cell proliferation in clear cell renal cell carcinoma. *Oncotarget*. 2016;7(19):27142-27157.

125. Fridman JS, Lowe SW. Control of apoptosis by p53. *Oncogene*. 2003;22(56):9030-9040.

126. Velez J, Enciso LJ, Suarez M, et al. Platelets promote mitochondrial uncoupling and resistance to apoptosis in leukemia cells: a novel paradigm for the bone marrow microenvironment. *Cancer Microenviron*. 2014;7(1-2):79-90.

127. Bottsford-Miller J, Choi HJ, Dalton HJ, et al. Differential platelet levels affect response to taxane-based therapy in ovarian cancer. *Clin Cancer Res*. 2015;21(3):602-610.

128. Kuter DJ. Managing thrombocytopenia associated with cancer chemotherapy. *Oncology (Williston Park)*. 2015;29(4):282-294.

129. Möhle R, Green D, Moore MA, Nachman RL, Rafii S. Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. *Proc Natl Acad Sci USA*. 1997;94(2):663-668.

130. Battinelli EM, Markens BA, Kulenthirarajan RA, Machlus KR, Flaumenhaft R, Italiano JE Jr. Anticoagulation inhibits tumor cell-mediated release of platelet angiogenic proteins and diminishes platelet angiogenic response. *Blood*. 2014;123(1):101-112.

131. Farooqi AA, Siddik ZH. Platelet-derived growth factor (PDGF) signalling in cancer: rapidly emerging signalling landscape. *Cell Biochem Funct*. 2015;33(5):257-265.

132. Johnson KE, Forward JA, Tippy MD, et al. Tamoxifen directly inhibits platelet angiogenic potential and platelet-mediated metastasis. *Arterioscler Thromb Vasc Biol*. 2017;37(4):664-674.

133. Zaslavsky A, Baek KH, Lynch RC, et al. Platelet-derived thrombospondin-1 is a critical negative regulator and potential biomarker of angiogenesis. *Blood*. 2010;115(22):4605-4613.

134. Wang Z, Huang H. Platelet factor-4 (CXCL4/PF-4): an angiostatic chemokine for cancer therapy. *Cancer Lett*. 2013;331(2):147-153.

135. Kamykowski J, Carlton P, Sehgal S, Storrie B. Quantitative immunofluorescence mapping reveals little functional coclustering of proteins within platelet α-granules. *Blood*. 2011;118(5):1370-1373.

136. Heijnen H, van der Sluijs P. Platelet secretory behaviour: as diverse as the granules … or not? *J Thromb Haemost*. 2015;13(12):2141-2151.

137. Peterson JE, Zurakowski D, Italiano JE Jr, et al. VEGF, PF4 and PDGF are elevated in platelets of colorectal cancer patients. *Angiogenesis*. 2012;15(2):265-273.

138. Zimmerman GA, Weyrich AS. Signal-dependent protein synthesis by activated platelets: new pathways to altered phenotype and function. *Arterioscler Thromb Vasc Biol*. 2008;28(3):s17-s24.

139. Klement GL, Yip TT, Cassiola F, et al. Platelets actively sequester angiogenesis regulators. *Blood*. 2009;113(12):2835-2842.

140. Kuznetsov HS, Marsh T, Markens BA, et al. Identification of luminal breast cancers that establish a tumor-supportive macroenvironment defined by proangiogenic platelets and bone marrow-derived cells. *Cancer Discov*. 2012;2(12):1150-1165.

141. Ho-Tin-Noé B, Goerge T, Cifuni SM, Duerschmied D, Wagner DD. Platelet granule secretion continuously prevents intratumor hemorrhage. *Cancer Res*. 2008;68(16):6851-6858.

142. Cervi D, Yip TT, Bhattacharya N, et al. Platelet-associated PF-4 as a biomarker of early tumor growth. *Blood*. 2008;111(3):1201-1207.

143. Pilatova K, Greplova K, Demlova R, Bencsikova B, Klement GL, Zdrazilova-Dubska L. Role of platelet chemokines, PF-4 and CTAP-III, in cancer biology. *J Hematol Oncol*. 2013;6(1):42.

144. Talmadge JE, Fidler IJ. AACR centennial series: the biology of cancer metastasis: historical perspective. *Cancer Res*. 2010;70(14):5649-5669.

145. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest*. 2009;119(6):1420-1428.

146. Labelle M, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. *Cancer Cell*. 2011;20(5):576-590.

147. Nakanishi M, Rosenberg DW. Multifaceted roles of PGE2 in inflammation and cancer. *Semin Immunopathol*. 2013;35(2):123-137.

148. Dovizio M, Maier TJ, Alberti S, et al. Pharmacological inhibition of platelet-tumor cell cross-talk prevents platelet-induced overexpression of cyclooxygenase-2 in HT29 human colon carcinoma cells. *Mol Pharmacol*. 2013;84(1):25-40.

149. Guillem-Llobat P, Dovizio M, Bruno A, et al. Aspirin prevents colorectal cancer metastasis in mice by splitting the crosstalk between platelets and tumor cells. *Oncotarget*. 2016;7(22):32462-32477.

150. Burkhalter RJ, Westfall SD, Liu Y, Stack MS. Lysophosphatidic acid initiates epithelial to mesenchymal transition and induces β-catenin-mediated transcription in epithelial ovarian carcinoma. *J Biol Chem*. 2015;290(36):22143-22154.

151. Ha JH, Ward JD, Radhakrishnan R, Jayaraman M, Song YS, Dhanasekaran DN. Lysophosphatidic acid stimulates epithelial to mesenchymal transition marker Slug/Snail2 in ovarian cancer cells via Gαi2, Src, and HIF1α signaling nexus. *Oncotarget*. 2016;7(25):37664-37679.

152. Khalid A, Wolfram J, Ferrari I, et al. Recent advances in discovering the role of CCL5 in metastatic breast cancer. *Mini Rev Med Chem*. 2015;15(13):1063-1072.

153. Karnoub AE, Dash AB, Vo AP, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature*. 2007;449(7162):557-563.

154. Halama N, Zoernig I, Berthel A, et al. Tumoral immune cell exploitation in colorectal cancer metastases can be targeted effectively by anti-CCR5 therapy in cancer patients. *Cancer Cell*. 2016;29(4):587-601.

155. von Hundelshausen P, Weber KS, Huo Y, et al. RANTES deposition by platelets triggers monocyte arrest on inflamed and atherosclerotic endothelium. *Circulation*. 2001;103(13):1772-1777.

156. Gilat D, Hershkoviz R, Mekori YA, Vlodavsky I, Lider O. Regulation of adhesion of CD4+ T lymphocytes to intact or heparinase-treated subendothelial extracellular matrix by diffusible or anchored RANTES and MIP-1 beta. *J Immunol*. 1994;153(11):4899-4906.

157. Pascual G, Avgustinova A, Mejetta S, et al. Targeting metastasis-initiating cells through the fatty acid receptor CD36. *Nature*. 2017;541(7635):41-45.

158. Camerer E, Qazi AA, Duong DN, Cornelissen I, Advincula R, Coughlin SR. Platelets, protease-activated receptors, and fibrinogen in hematogenous metastasis. *Blood*. 2004;104(2):397-401.

159. Egan K, Cooke N, Kenny D. Living in shear: platelets protect cancer cells from shear induced damage. *Clin Exp Metastasis*. 2014;31(6):697-704.

160. Zhao L, Thorsheim CL, Suzuki A, et al. Phosphatidylinositol transfer protein-α in platelets is inconsequential for thrombosis yet is utilized for tumor metastasis. *Nat Commun*. 2017;8(1):1216.

161. Lopes-Bastos BM, Jiang WG, Cai J. Tumour-endothelial cell communications: important and indispensable mediators of tumour angiogenesis. *Anticancer Res*. 2016;36(3):1119-1126.

162. Qian BZ, Li J, Zhang H, et al. CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. *Nature*. 2011;475(7355):222-225.

163. Qian B, Deng Y, Im JH, et al. A distinct macrophage population mediates metastatic breast cancer cell extravasation, establishment and growth. *PLoS One*. 2009;4(8):e6562.

164. Boucharaba A, Serre CM, Grès S, et al. Platelet-derived lysophosphatidic acid supports the progression of osteolytic bone metastases in breast cancer. *J Clin Invest*. 2004;114(12):1714-1725.

165. Boucharaba A, Serre CM, Guglielmi J, Bordet JC, Clézardin P, Peyruchaud O. The type 1 lysophosphatidic acid receptor is a target for therapy in bone metastases. *Proc Natl Acad Sci USA*. 2006;103(25):9643-9648.

166. Leblanc R, Lee SC, David M, et al. Interaction of platelet-derived autotaxin with tumor integrin αVβ3 controls metastasis of breast cancer cells to bone. *Blood*. 2014;124(20):3141-3150.

167. Dunn GP, Dunn IF, Curry WT. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human glioma. *Cancer Immun*. 2007;7:12.

168. Jiang D, Liu Y, Wang H, et al. Tumour infiltrating lymphocytes correlate with improved survival in patients with esophageal squamous cell carcinoma. *Sci Rep*. 2017;7:44823.

169. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*. 2006;313(5795):1960-1964.

170. Fridman WH, Galon J, Pagès F, Tartour E, Sautès-Fridman C, Kroemer G. Prognostic and predictive impact of intra- and peritumoral immune infiltrates. *Cancer Res*. 2011;71(17):5601-5605.

171. Blank CU, Haanen JB, Ribas A, Schumacher TN. Cancer immunology. The "cancer immunogram". *Science*. 2016;352(6286):658-660.

172. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature*. 2008;454(7203):436-444.

173. Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol*. 2017;14(7):399-416.

174. Adeegbe DO, Nishikawa H. Natural and induced T regulatory cells in cancer. *Front Immunol*. 2013;4:190.

175. Teng MW, Galon J, Fridman WH, Smyth MJ. From mice to humans: developments in cancer immunoediting. *J Clin Invest*. 2015;125(9):3338-3346.

176. Couzin-Frankel J. Breakthrough of the year 2013. Cancer immunotherapy. *Science*. 2013;342(6165):1432-1433.

177. Crusz SM, Balkwill FR. Inflammation and cancer: advances and new agents. *Nat Rev Clin Oncol*. 2015;12(10):584-596.

178. Nieswandt B, Hafner M, Echtenacher B, Männel DN. Lysis of tumor cells by natural killer cells in mice is impeded by platelets. *Cancer Res*. 1999;59(6):1295-1300.

179. Palumbo JS, Talmage KE, Massari JV, et al. Platelets and fibrin(ogen) increase metastatic potential by impeding natural killer cell-mediated elimination of tumor cells. *Blood*. 2005;105(1):178-185.

180. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. *Annu Rev Immunol*. 2004;22(1):329-360.

181. Bauer S, Groh V, Wu J, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science*. 1999;285(5428):727-729.

182. Kopp HG, Placke T, Salih HR. Platelet-derived transforming growth factor-beta down-regulates NKG2D thereby inhibiting natural killer cell antitumor reactivity. *Cancer Res*. 2009;69(19):7775-7783.

183. Viel S, Marçais A, Guimaraes FS, et al. TGF-β inhibits the activation and functions of NK cells by repressing the mTOR pathway. *Sci Signal*. 2016;9(415):ra19.

184. Placke T, Örgel M, Schaller M, et al. Platelet-derived MHC class I confers a pseudonormal phenotype to cancer cells that subverts the antitumor reactivity of natural killer immune cells. *Cancer Res*. 2012;72(2):440-448.

185. Tímár J, Tóvári J, Rásó E, Mészáros L, Bereczky B, Lapis K. Platelet-mimicry of cancer cells: epiphenomenon with clinical significance. *Oncology*. 2005;69(3):185-201.

186. Placke T, Salih HR, Kopp HG. GITR ligand provided by thrombopoietic cells inhibits NK cell antitumor activity. *J Immunol*. 2012;189(1):154-160.

187. Chen M, Geng JG. P-selectin mediates adhesion of leukocytes, platelets, and cancer cells in inflammation, thrombosis, and cancer growth and metastasis. *Arch Immunol Ther Exp (Warsz)*. 2006;54(2):75-84.

188. Simon DI, Chen Z, Xu H, et al. Platelet glycoprotein ibalpha is a counterreceptor for the leukocyte integrin Mac-1 (CD11b/CD18). *J Exp Med*. 2000;192(2):193-204.

189. Demers M, Wagner DD. Neutrophil extracellular traps: a new link to cancer-associated thrombosis and potential implications for tumor progression. *OncoImmunology*. 2013;2(2):e22946.

190. Park J, Wysocki RW, Amoozgar Z, et al. Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps. *Sci Transl Med*. 2016;8(361):361ra138.

191. Mayadas TN, Johnson RC, Rayburn H, Hynes RO, Wagner DD. Leukocyte rolling and extravasation are severely compromised in P selectin-deficient mice. *Cell*. 1993;74(3):541-554.

192. Hoffmeister KM, Felbinger TW, Falet H, et al. The clearance mechanism of chilled blood platelets. *Cell*. 2003;112(1):87-97.

193. Metelli A, Wu BX, Fugle CW, et al. Surface expression of TGFβ docking receptor GARP promotes oncogenesis and immune tolerance in breast cancer. *Cancer Res*. 2016;76(24):7106-7117.

194. Rachidi S, Metelli A, Riesenberg B, et al. Platelets subvert T cell immunity against cancer via GARP-TGFβ axis. *Sci Immunol*. 2017;2(11).

195. Michael JV, Wurtzel JGT, Mao GF, et al. Platelet microparticles infiltrating solid tumors transfer miRNAs that suppress tumor growth. *Blood*. 2017;130(5):567-580.

196. Wang C, Sun W, Ye Y, Hu Q, Bomba HN, Gu Z. In situ activation of platelets with checkpoint inhibitors for post-surgical cancer immunotherapy. *Nat Biomed Eng*. 2017;1:0011.

197. Flossmann E, Rothwell PM; British Doctors Aspirin Trial and the UK-TIA Aspirin Trial. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. *Lancet*. 2007;369(9573):1603-1613.

198. Rothwell PM, Wilson M, Elwin CE, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. *Lancet*. 2010;376(9754):1741-1750.

199. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. *Lancet*. 2011;377(9759):31-41.

200. Rothwell PM, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. *Lancet*. 2012;379(9826):1591-1601.

201. Rothwell PM, Price JF, Fowkes FG, et al. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. *Lancet*. 2012;379(9826):1602-1612.

202. Thun MJ, Jacobs EJ, Patrono C. The role of aspirin in cancer prevention. *Nat Rev Clin Oncol*. 2012;9(5):259-267.

203. Nan H, Hutter CM, Lin Y, et al; GECCO. Association of aspirin and NSAID use with risk of colorectal cancer according to genetic variants. *JAMA*. 2015;313(11):1133-1142.

204. Friis S, Riis AH, Erichsen R, Baron JA, Sørensen HT. Low-dose aspirin or nonsteroidal anti-inflammatory drug use and colorectal cancer risk: a population-based, case-control study. *Ann Intern Med*. 2015;163(5):347-355.

205. Piepoli MF, Hoes AW, Agewall S, et al; ESC Scientific Document Group. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: the Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J*. 2016;37(29):2315-2381.

206. De Berardis G, Sacco M, Strippoli GF, et al. Aspirin for primary prevention of cardiovascular events in people with diabetes: meta-analysis of randomised controlled trials. *BMJ*. 2009;339:b4531.

207. Elwood P, Morgan G. The harms of low-dose aspirin prophylaxis are overstated. *Ann Oncol*. 2015;26(2):441-442.

208. Patrignani P, Patrono C. Aspirin and cancer. *J Am Coll Cardiol*. 2016;68(9):967-976.

209. Cuzick J, Thorat MA, Bosetti C, et al. Estimates of benefits and harms of prophylactic use of aspirin in the general population. *Ann Oncol*. 2015;26(1):47-57.

210. Choi J, Ghoz HM, Peeraphatdit T, et al. Aspirin use and the risk of cholangiocarcinoma. *Hepatology*. 2016;64(3):785-796.

211. Bosetti C, Rosato V, Gallus S, La Vecchia C. Aspirin and urologic cancer risk: an update. *Nat Rev Urol*. 2012;9(2):102-110.

212. Zhong S, Chen L, Zhang X, Yu D, Tang J, Zhao J. Aspirin use and risk of breast cancer: systematic review and meta-analysis of observational studies. *Cancer Epidemiol Biomarkers Prev*. 2015;24(11):1645-1655.

213. Zhang D, Bai B, Xi Y, Wang T, Zhao Y. Is aspirin use associated with a decreased risk of ovarian cancer? A systematic review and meta-analysis of observational studies with dose-response analysis. *Gynecol Oncol*. 2016;142(2):368-377.

214. Shiao J, Thomas KM, Rahimi AS, et al. Aspirin/antiplatelet agent use improves disease-free survival and reduces the risk of distant metastases in stage II and III triple-negative breast cancer patients. *Breast Cancer Res Treat*. 2017;161(3):463-471.

215. Verdoodt F, Friis S, Dehlendorff C, Albieri V, Kjaer SK. Non-steroidal anti-inflammatory drug use and risk of endometrial cancer: a systematic review and meta-analysis of observational studies. *Gynecol Oncol*. 2016;140(2):352-358.

216. Jiang MJ, Dai JJ, Gu DN, Huang Q, Tian L. Aspirin in pancreatic cancer: chemopreventive effects and therapeutic potentials. *Biochim Biophys Acta*. 2016;1866(2):163-176.

217. Langley RE, Rothwell PM. Aspirin in gastrointestinal oncology: new data on an old friend. *Curr Opin Oncol*. 2014;26(4):441-447.

218. Kurtova AV, Xiao J, Mo Q, et al. Blocking PGE2-induced tumour repopulation abrogates bladder cancer chemoresistance. *Nature*. 2015;517(7533):209-213.

219. Zelenay S, van der Veen AG, Böttcher JP, et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. *Cell*. 2015;162(6):1257-1270.

220. Todoric J, Antonucci L, Karin M. Targeting inflammation in cancer prevention and therapy. *Cancer Prev Res (Phila)*. 2016;9(12):895-905.

221. Chan AT, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. *N Engl J Med*. 2007;356(21):2131-2142.

222. Sostres C, Gargallo CJ, Lanas A. Aspirin, cyclooxygenase inhibition and colorectal cancer. *World J Gastrointest Pharmacol Ther*. 2014;5(1):40-49.

223. Eikelboom JW, Hirsh J, Spencer FA, Baglin TP, Weitz JI. Antiplatelet Drugs: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 2012;141(2 suppl):e89S-e119S.

224. Mitrugno A, Sylman JL, Ngo AT, et al. Aspirin therapy reduces the ability of platelets to promote colon and pancreatic cancer cell proliferation: implications for the oncoprotein c-MYC. *Am J Physiol Cell Physiol*. 2017;312(2):C176-C189.

225. Sitia G, Aiolfi R, Di Lucia P, et al. Antiplatelet therapy prevents hepatocellular carcinoma and improves survival in a mouse model of chronic hepatitis B. *Proc Natl Acad Sci USA*. 2012;109(32):E2165-E2172.

226. Avivi D, Moshkowitz M, Detering E, Arber N. The role of low-dose aspirin in the prevention of colorectal cancer. *Expert Opin Ther Targets*. 2012;16(sup1 Suppl 1):S51-S62.

227. Dovizio M, Alberti S, Sacco A, et al. Novel insights into the regulation of cyclooxygenase-2 expression by platelet-cancer cell cross-talk. *Biochem Soc Trans*. 2015;43(4):707-714.

228. Dixon DA, Tolley ND, Bemis-Standoli K, et al. Expression of COX-2 in platelet-monocyte interactions occurs via combinatorial regulation involving adhesion and cytokine signaling. *J Clin Invest*. 2006;116(10):2727-2738.

229. Patrono C, Patrignani P, García Rodríguez LA. Cyclooxygenase-selective inhibition of prostanoid formation: transducing biochemical selectivity into clinical read-outs. *J Clin Invest*. 2001;108(1):7-13.

230. Santilli F, Boccatonda A, Davì G. Aspirin, platelets, and cancer: The point of view of the internist. *Eur J Intern Med*. 2016;34:11-20.

231. Voora D, Rao AK, Jalagadugula GS, et al. Systems pharmacogenomics finds RUNX1 is an aspirin-responsive transcription factor linked to cardiovascular disease and colon cancer. *EBioMedicine*. 2016;11:157-164.

232. Alfonso L, Ai G, Spitale RC, Bhat GJ. Molecular targets of aspirin and cancer prevention. *Br J Cancer*. 2014;111(1):61-67.

233. Yiannakopoulou E. Targeting epigenetic mechanisms and microRNAs by aspirin and other non steroidal anti-inflammatory agents–implications for cancer treatment and chemoprevention. *Cell Oncol (Dordr)*. 2014;37(3):167-178.

234. Usman MW, Luo F, Cheng H, Zhao JJ, Liu P. Chemopreventive effects of aspirin at a glance. *Biochim Biophys Acta*. 2015;1855(2):254-263.

235. Zigler M, Kamiya T, Brantley EC, Villares GJ, Bar-Eli M. PAR-1 and thrombin: the ties that bind the microenvironment to melanoma metastasis. *Cancer Res*. 2011;71(21):6561-6566.

236. Cho MS, Noh K, Haemmerle M, et al. Role of ADP receptors on platelets in the growth of ovarian cancer. *Blood*. 2017;130(10):1235-1242.

237. Kolandaivelu K, Bhatt DL. Novel antiplatelet therapies. In: Michelson AD, ed. Platelets. London: Academic Press; 2013:1185-1213.

238. Mammadova-Bach E, Ollivier V, Loyau S, et al. Platelet glycoprotein VI binds to polymerized fibrin and promotes thrombin generation. *Blood*. 2015;126(5):683-691.

239. Alshehri OM, Hughes CE, Montague S, et al. Fibrin activates GPVI in human and mouse platelets. *Blood*. 2015;126(13):1601-1608.

240. Jain S, Russell S, Ware J. Platelet glycoprotein VI facilitates experimental lung metastasis in syngenic mouse models. *J Thromb Haemost*. 2009;7(10):1713-1717.

241. Erpenbeck L, Schön MP. Deadly allies: the fatal interplay between platelets and metastasizing cancer cells. *Blood*. 2010;115(17):3427-3436.

242. Romo GM, Dong JF, Schade AJ, et al. The glycoprotein Ib-IX-V complex is a platelet counterreceptor for P-selectin. *J Exp Med*. 1999;190(6):803-814.

243. Singh J, Zeller W, Zhou N, et al. Structure-activity relationship studies leading to the identification of (2E)-3-[l-[(2,4-dichlorophenyl) methyl]-5-fluoro-3-methyl-lH-indol-7-yl]-N-[(4,5-dichloro-2-thienyl)sulfonyl]-2-propenamide (DG-041), a potent and selective prostanoid EP3 receptor antagonist, as a novel antiplatelet agent that does not prolong bleeding. *J Med Chem*. 2010;53(1):18-36.

244. Patil S, Newman DK, Newman PJ. Platelet endothelial cell adhesion molecule-1 serves as an inhibitory receptor that modulates platelet responses to collagen. *Blood*. 2001;97(6):1727-1732.

245. Kahale LA, Hakoum MB, Tsolakian IG, et al. Oral anticoagulation in people with cancer who have no therapeutic or prophylactic indication for anticoagulation. *Cochrane Database Syst Rev*. 2017;12:CD006466.

246. Yao Y, Chen Y, Adili R, et al. Plant-based food cyanidin-3-glucoside modulates human platelet glycoprotein VI signaling and inhibits platelet activation and thrombus formation. *J Nutr*. 2017;147(10):1917-1925.

247. Yang Y, Shi Z, Reheman A, et al. Plant food delphinidin-3-glucoside significantly inhibits platelet activation and thrombosis: novel protective roles against cardiovascular diseases. *PLoS One*. 2012;7(5):e37323.

248. Li J, Wang Y, Yucel Y, Ni H. Platelet desialylation: novel mechanism of immune tolerance. *Res Pract Thromb Haemost*. 2017;1(suppl 1):248.

249. Serebruany VL, Cherepanov V, Cabrera-Fuentes HA, Kim MH. Solid cancers after antiplatelet therapy: Confirmations, controversies, and challenges. *Thromb Haemost*. 2015;114(6):1104-1112.

250. Serebruany V, Floyd J, Chew D. Excess of solid cancers after prasugrel: the Food and Drug Administration outlook [published online ahead of print 10 July 2010]. *Am J Ther*. doi:10.1097/MJT.0b013e3181e9b675.

251. Serebruany VL, Cherepanov V, Golukhova EZ, Kim MH. The Dual Antiplatelet Therapy Trial after the FDA update: non-cardiovascular deaths, cancer and optimal treatment duration. *Cardiology*. 2015;132(2):74-80.

252. Mauri L, Kereiakes DJ, Yeh RW, et al; DAPT Study Investigators. Twelve or 30 months of dual antiplatelet therapy after drug-eluting stents. *N Engl J Med*. 2014;371(23):2155-2166.

253. Serebruany VL. Aggressive chronic platelet inhibition with prasugrel and increased cancer risks: revising oral antiplatelet regimens? *Fundam Clin Pharmacol*. 2009;23(4):411-417.