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Repurposing Antiplatelet Drugs to Treat Platelet Mediated Metastasis

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REPUROSING ANTIPLATELET DRUGS TO TREAT PLATELET MEDIATED METASTASIS

By

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DEDICATION

My thesis is dedicated to my family, friends, and anyone else that has helped encourage me through the thesis process. You all helped keep me focused and remind me of the importance of keeping my eyes on my goals. I can never thank you all enough.

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ABSTRACT

BRIANNA NICOLE CHAMBERS: Repurposing Antiplatelet Drugs to Treat Platelet Mediated Metastasis (Under the direction of Dr. Thomas Werfel)

By applying cardiovascular drugs, which already have been proven effective and safe, to other diseases we can speed the drug discovery process, specifically to translate them into treatments for platelet-mediated metastasis. Each cardiovascular drug within antiplatelet drugs, targets one of the four following pathways: COX-1, thromboxane, P2Y12, or thrombin. There has been varying levels of success with treating adenocarcinomas like breast and colorectal cancer. Continued success in treatment could be the result of pharmacogenetics and further evaluation of dosage levels. By isolating mutation rates in various adenocarcinomas there is the possibility of a targeted treatment based on the mutated gene.

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Introduction

Antithrombotic drugs can be classified in three major categories: antiplatelets, anticoagulants, and fibrinolytics. Each of these types of drugs works to prevent clot formation though each has a unique mechanism of action. Platelet aggregation is specifically targeted by the antiplatelet drugs. However, anticoagulants target the clotting cascade and fibrinolytic drugs dissolve blood clots that have already formed.

Antiplatelet drugs prevent the aggregation of blood platelets which helps reduce blood clot formation. Platelets are blood cells with the purpose of preventing hemorrhage after injury or trauma to a blood vessel [1]. Repair is initiated after platelet activation causes a shape change, adhesion, and a release of biologically active factors to repair the damaged tissue [1]. Some of the factors released for thrombosis include prothrombin, fibrinogen, ADP, and TXA2 [1]. Antiplatelet drugs are commonly prescribed to treat cardiovascular conditions such as: predicted thickening in post-coronary episodes, insecure angina, ischemic strokes, and transient ischemic assaults (TIA). Aspirin helps prevent atherosclerosis by increasing vasodilation and reducing thrombosis in blood vessels. There does not have to be a large blockage in a vein for an antiplatelet to be prescribed to a patient, it can be preventative to help avoid a plaque from forming with the antiplatelet agents and dual antiplatelet therapy[2]. The most commonly prescribed antiplatelets are as follows: aspirin, clopidogrel (Plavix), dipyridamole (Persantine), prasugrel (Effient), and ticagrelor (Brilinta) [2].

Anticoagulants prevent the blood from clotting by blocking the clotting cascade. Anticoagulants are often referred to incorrectly as blood thinners although these medications do not change the viscosity of the blood or break down pre-existing blood clots. Instead, by blocking the clotting cascade there is a lower chance of a blood clot formation. These drugs are often used in cardiovascular medicine to keep destructive clusters from altering vein shape, keep clots from expanding or causing major issues, and preventatively for a stroke or intermittent stroke. The most commonly prescribed anticoagulants are apixaban (Eliquis), dabigatran (Pradaxa), edoxaban (Savaysa), heparin (various), rivaroxaban (Xarelto), and warfarin (Coumadin) [2]. Anticoagulants are the most commonly prescribed medications in the world due to their success in the treatment of cardiovascular disease [2].

Fibrinolytic drugs, also referred to as thrombolytic drugs or plasminogen activators, destroy clots that have already been formed. There are three unique drugs that can dissolve clots: streptokinase (SK), urokinase (UK), or tissue plasminogen activator (tPA) [3]. The fibrinolytic drugs are used to remove clots that have already formed as compared to anticoagulants, which target the clotting cascade, and antiplatelets, which target platelet aggregation, both of which prevent a clot from forming. The streptokinase, a subset of fibrinolytic drugs, is given intravenously post-myocardial obstruction and it helps restore the blood flow to the heart and nearby vessels [3]. Additionally, SK helps limit the amount of tissue death in the heart and surrounding tissues following myocardial infarction.

Cancer metastasis is a common process that occurs when cancer from the primary organ spreads to secondary and tertiary sites throughout the body. Platelets play a vital role in the multi-step process of cancer metastasis; therefore, antiplatelet drugs may be useful in limiting metastasis of cancers (**Figure 1**). The basic steps of metastasis are as follows: tumor cell growth in local tissue, intravasation of tumor cells into the bloodstream, cell survival in the circulatory system, extravasation of tumor cells out of the bloodstream, and colonization in a secondary location [4]. Platelets influence the steps leading up to metastasis by altering the tumor microenvironment and transitioning tumor cells to a more migratory phenotype [5]. Once entering the circulatory system, platelets can support metastasis by improving tumor cell survival, hiding tumor cells from the immune system, aiding in tumor cell arrest and adhesion, and increasing tumor cell extravasation (**Figure 1**). Because platelets encourage tumor survival, arrest and adhesion, and tumor intravasation and extravasation, it is exciting to speculate that antiplatelet drugs could be repurposed from cardiovascular medicine to prevent metastasis of solid cancers with potentially transformative results.

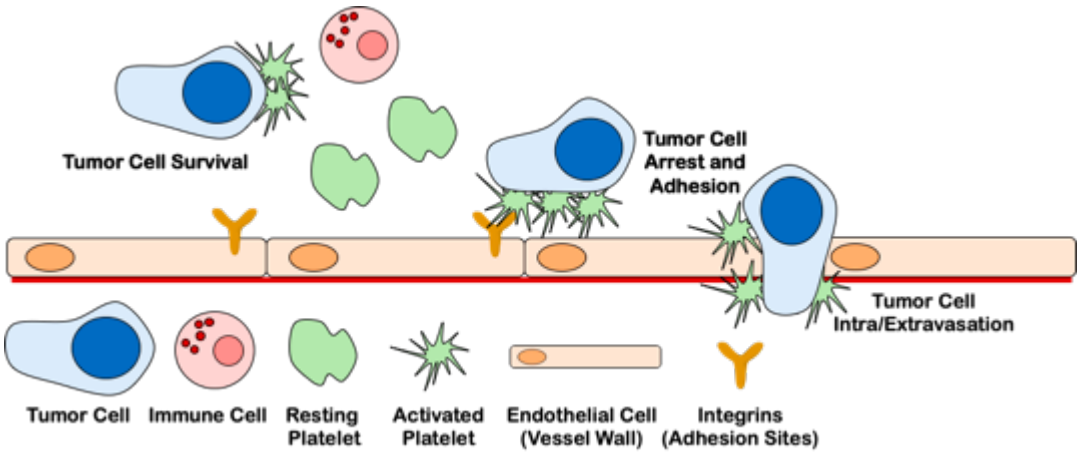

Figure 1. Schematic representation of platelet-mediated metastasis. Platelets interact with tumor cells as they spread through the blood, contributing to metastasis by supporting tumor cell survival, arrest and adhesion of tumor cells to blood vessel walls, and escape (extravasation) of tumor cells from the blood into new tissues.

Chapter 1

Four molecular targets are common in platelet activation and could be targeted to reduce metastasis: cyclooxygenase (COX), thromboxane A2 receptor (TBXA2R), P2Y12, and protease-activated receptors (PARs). Each of these actionable molecular targets offers a unique opportunity for treatment based on the availability of existing cardiovascular drugs and the prominence of each molecule in different types of cancer. **Figure 2** describes an overview of signaling related to each of the four targets.

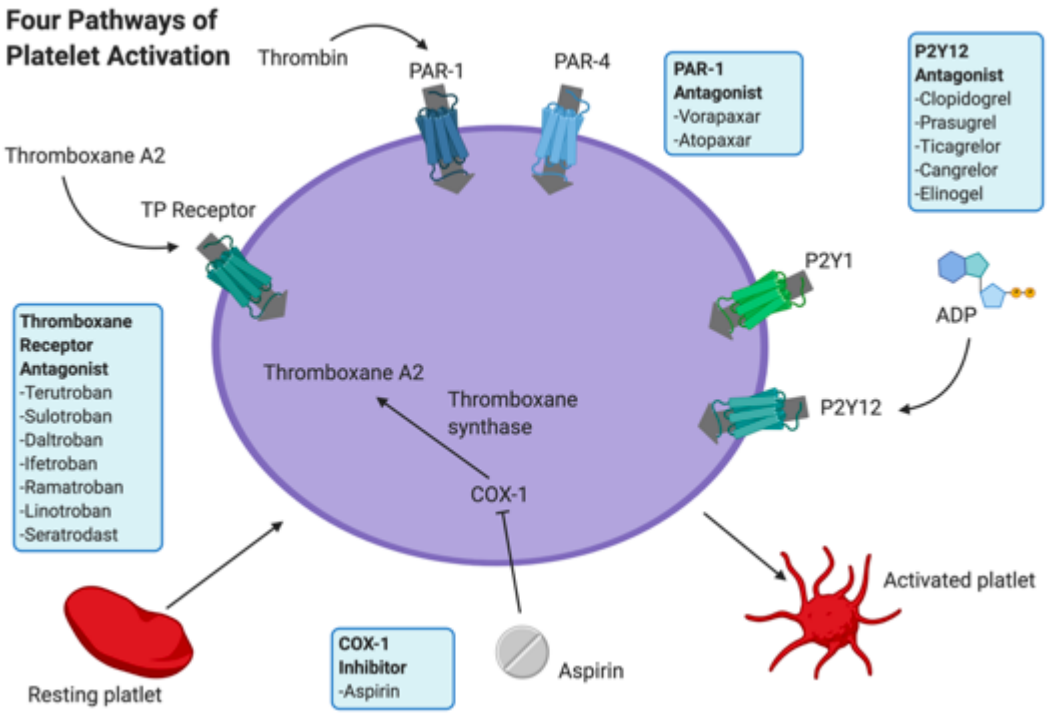

Figure 2. Overview of four platelet signaling pathways reviewed here: Cyclooxygenase, Thromboxane, P2Y12, and Thrombin. Each of these pathways could

potentiate cancer metastasis and thus could serve as a therapeutic target to block metastasis and lower cancer mortality.

Aspirin/Cyclooxygenase Pathway

The first non-steroidal anti-inflammatory drug (NSAID) discovered was aspirin, and it was one of the first modern drugs in common use. The mechanism of aspirin's pain relief properties, specifically the molecular mechanism of prophylaxis of coronary occlusion, was determined during the twentieth century [6]. By the end of the twentieth century, there were new reports that taking a low-dosage aspirin daily can prevent heart attack and stroke. Both heart attacks and strokes are caused by a blockage in blood vessels leading to the heart and brain, respectively. Taking low-dose aspirin helps prevent the formation of blood clots and thus helps reduce the likelihood of a heart attack [7]. The mechanism of action of aspirin is mediated via the cyclooxygenase signaling pathway. Cyclooxygenase-1 (COX-1) plays a role in the synthesis of prostaglandins (PG) and thromboxane A2 (TXA2). Aspirin inhibits COX-1 by irreversibly acetylating a serine residue, which prevents the binding of arachidonic acid to COX-1 [8].

It is now appreciated that aspirin has multifaceted effects on cancer and therefore recent studies have assessed the role of COX-signaling on tumor development and progression. Aspirin can reduce cancer by induction of caspases [9], upregulation of anti-apoptotic protein Bax, downregulation of apoptosis suppressor Bcl2, and suppression of pro-angiogenic factor VEGF [10]. Aspirin-induced inhibition of epithelial-mesenchymal

transition (EMT) occurs in SK-OV-3 ovarian cancer cell lines [11] and in HT-29 human colon carcinoma cell lines [12]. The EMT reduction results from E-cadherin downregulation and Twist1 upregulation [11]. Other mechanisms include selective reactive oxygen species (ROS) formation only in cancer cells [13], strong inhibition of NF-κβ leading to apoptosis [14] and reduction of phosphorylated Stat-3 and Erk1/2 [14].

Aspirin also targets cancer cell migration and invasion. Fibromodulin is an extracellular matrix protein which is also a downstream protein transcriptionally regulated by β-catenin complexed with other molecules. In normal situations, Histone deacetylase 6 (HDAC6) deacetylates β-catenin resulting in its dephosphorylation and nuclear translocation. Aspirin directly inhibits HDAC6, causing phosphorylation and degradation of β-catenin indirectly affecting fibromodulin. As fibromodulin plays an important role in breast cancer cell motility via Erk activation, indirect inhibition of fibromodulin mediated by aspirin results in decreased motility [15]. This study identifies the importance of fibromodulin in breast cancer, and elucidates the mechanism of aspirin inhibition of the pathway [15].

Platelet-mediated cancer metastasis was inhibited by aspirin in animal models of lung metastasis in a study by Lucotti et al in 2019 [16]. The long-term administration of aspirin revealed the inhibition of the COX-1 pathway and the product TXA2 resulted in reduced metastasis [16]. This study specifically discovered that COX-1 and TXA2 play a critical role in the transition of tumor cells to the vascular system [16].

TBXA2R Antagonists

TXA2, a platelet agonist, is derived from platelets and drives platelet activation by binding of the TBXA2R [17]. TXA2 activated platelets are vital in the formation of blood clots. Thromboxane synthase inhibitors (TXSIs) limit TXA2 formation and increase production of antiaggregatory prostaglandins PGI2 and PGD2. Alternatively, TXA2 receptor antagonists (TXRA) reduce the action of TXA2 and PGH2 on platelets and vessel walls [18]. TXSI and TXRA are more sensitive compared to aspirin because they have more specific activity in the TXA2 pathway, and bypass portions of the cyclooxygenase signaling pathway. TXSI prevents the conversion of PGH2 to TXA2, lowering the production of TXA2 in platelets, while TXRA directly blocks TBXA2R.

Lung cancer is one of the most deadly malignant cancers in the Western world, and more individuals die from lung cancer than breast, colon, and prostate cancer combined [19]. A study of 48 samples from non-small cell lung cancer (NSCLC) patients with matching healthy lung tissues revealed elevated COX-isoenzymes and terminal synthases of prostanoid synthesis [20]. Expanded COX-2 and concurrent down-regulation of COX-1 in NSCLC were identified further by immunohistochemistry [20]. There were elevated levels of TXB2, a metabolite of TXA2, observed in human lung cancer tissues which suggests expanded TXA2S expression in lung cancer [21]. Expression of TXA2S, COX-1, COX-2, and microsomal prostaglandin-E synthase were overall higher in metastatic cases of NSCLC when compared to non-metastatic cases [22].

In recent molecular studies, TBXA2R expression was reported in five of six NSCLC cell lines [23]. Looking at A549 cells with abnormal TBXA2R expression showed noticeably more tumor development and expanded vascularization than the control A549 when placed in recipient mice [23]. Although COX-2 overexpression has been observed in lung tumors, there is little understanding of the factors that control the process. Through activation of TBXA2R and the mimetic of TXA2, IBOP, it initiates COX-2 through four distinct pathways: extracellular signal-regulated kinase (ERK), p38 MAPK, JAK, and β-catenin. Similarly, translation factors such as NFκB, cAMP response element binding (CREB), C/EBP, and Stat3 are downstream signaling molecules that interact with COX-2 and explain the TBXA2R-mediated expression of COX-2, which is significant because of the known roles of COX-2 in metastatic cancers [24].

There is evidence that strongly suggests that Nurr1 is instrumental for the proliferation of lung carcinoma cells (H157) through regulation of cyclin D1 expression [25]. Other studies have shown that TBXA2R activates Nurr1 expression by an epidermal growth factor receptor (EGFR)-independent mechanism. Oral administration of TXA2S inhibitors CI or furegrelate lessened the number and size of metastatic colonies in the lung after infusion of LLC cells [26]. With this evidence, it supports that TXA2S contributes to lung metastasis and TXA2S inhibitors are potential anti-metastatic agents.

Given the connection between smoking and lung cancer and the role of TXA2 signaling in lung cancer, it will be useful to analyze if TXA2 signaling decreases lung cancer caused by smoking. Studies have shown that malignant lung tissue of smokers has elevated TXB2 levels when compared to malignant lung tissue of non-smokers [27]. To investigate the causal role for TXA2 signaling in lung cancer, Huang et al. associated TXA2S, TXB2, and cancer-causing agent 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in smokers and non-smokers [28]. There were significantly higher levels of TXA2S expression in tumor tissue when compared to non-tumor tissue from similar patients. A connection to NNK could be assumed from the perception that less tumors from non-smokers had TXA2S expression when compared to tumors from smokers. All of the tissue from smokers, both tumor and non-tumor, were positive for TXA2S expression, but only one-quarter of non-smokers were positive for TXA2S expression. NNK increased TXA2S and TXA2 levels and TBXA2R expression both *in vivo* and in lung cancer cells *in vitro*. Additionally, TXA2 may cause CREB to activate through PI3K/Akt and ERK pathways, which causes the development of lung malignant growth by NNK [29]. Most importantly, TXA2S and TBXA2R inhibitors prevented development and promoted apoptosis of lung cancer cells [29] showing the significance of TXA2-to-NNK instigated tumor development in lung cancer.

Cathcart et al. investigated the expression of TXA2S in NSCLC in 204 patients, to determine if TXA2S was a prognostic as well as survival factor [30]. They analyzed the purpose of TXA2S in growth and migration of NSCLC cells *in vitro*. Although there was no medical role for TXA2S in NSCLC, they were able to confirm that plasma and protein

levels of TXA2S and TXB2 were higher overall in tumor tissues when compared to healthy tissues [30]. The setup of their model revealed that enhanced TXA2S expression advances proliferation, motility, and survival of cancer cells.

P2Y12 Receptor Antagonists

The P2Y12 inhibitors are part of the widely used class of antiplatelet drugs including: clopidogrel, ticlopidine, ticagrelor, prasugrel, and cangrelor. The receptor is activated by ADP (adenosine diphosphate) and results in platelet activation. ADP is a secondary agonist for platelets secreted from granules once activated by thrombin or collagen [31]. Extracellular nucleotides act on platelets through one of two purinergic receptors, adenosine (P1) or nucleotide (P2) receptor. The P2 receptors are ligand-gated ion channels (P2X) and G-protein coupled receptors (P2Y) [32]. Stimulating both G-coupled P2Y1 receptors (P2Y1-R) and G-coupled P2Y12 receptors (P2Y12-R) by extracellular ADP is necessary for platelet aggregation. Then, the formation of thrombus is dependent upon P2Y12-R activation [32]. Thienopyridines (ticlopidine, clopidogrel, prasugrel) use an irreversible mechanism to inhibit the P2Y12 receptor. Another class, cyclopentyl-triazolo-pyrimidine, uses reversible antagonists of the P2Y12 receptor and includes ticagrelor, cangrelor, and elinogrel. The receptor is a G-coupled protein receptor (GCPR), that inhibits the adenylate cyclase-mediated signaling pathway and decreases the intracellular cAMP levels [33]. ADP binds to the P2Y12 receptor and causes a number of intracellular signaling events downstream of the Gi pathway that contribute to fibrinogen receptor activation and platelet aggregation [34].

After aspirin, P2Y12 receptor antagonists are the most prescribed antiplatelet drugs for CVD. When a patient experiences a severe coronary event, the P2Y12 receptor antagonist is administered to prevent blood clots in patients after percutaneous coronary intervention (PCI).

In a case-control study in Spain, they found that the use of clopidogrel, a P2Y12 inhibitor, alone or in combination with aspirin, reduced CRC incidence by 20-30%, which were similar results to aspirin alone [35]. The first demonstration of inhibiting spontaneous metastasis occurred in 1981, when ticlopidine was shown to inhibit experimental pulmonary metastasis in a rodent model when injected with B16 melanoma cells or AH130 rat ascites hepatoma cells [36]. In 2013, P2Y12 deficient mice had reduced weight of lung metastasis not affecting the primary tumors under the same experimental conditions [37]. The hypothesis that the ADP receptor has a role in metastasis was further corroborated when platelets from P2Y12 deficient mice significantly reduced metastasis by Lewis lung carcinomas (LLC). Moreover, this P2Y12 deficiency leads to significant reduction of TGF-β in the tumor microenvironment and reduced EMT [37]. Further support that P2Y12 contributes to metastasis came from Guillem-Llobat et al., where they found that HT29 colon cancer cells cultured with platelets lead to the induction of EMT [38]. When the cells were grown with the P2Y12 inhibitor ticagrelor, E-cadherin and cell migration were both reduced, suggesting P2Y12 inhibition could reverse the platelet-mediated shift of tumor cells to an EMT phenotype [39].

Ticagrelor reduces the interaction of platelets with breast cancer cell lines MCF-7, MDA-MB-468, and MDA-MB-231 by reducing the ability of platelets to activate with ADP present [40]. MDA-MB-231 was shown to have the highest potential for metastasis, and as a result ticagrelor was most successful at reducing metastasis in this cell line [40]. There was no effect on the NK-cell mediated killing of cancer cells or cancer proliferation likely due to the reversibility of the binding. Even with little effect on primary tumor growth, there is improvement in survival due to decreased platelet cancer cell aggregates which metastasized to the lungs of untreated mice [40].

Previously considered restricted to platelets [41] , P2Y12-R has been found in many other locations like microglia [42], astrocytes [43], vascular smooth cells [43], eosinophils [44], and tumor-associated macrophages (TAMs) [45]. Despite these locations, the understanding of P2Y12-R expression in cancer cells is not well understood [32]. In astrocytes, part of brain cancer cells in glioblastoma, P2Y12-R has been detected to drive proliferative response [46]. However, MCF-7 and MDA-MB-231 breast cancer cells express P2Y12-R during stress conditions (e.g. in serum starvation, cisplatin treatment) [47], which suggest that P2Y12-R can play a role in chemotherapy resistance as well [47]. Moreover, with P2Y12 also being expressed in TAMs, it could also contribute to cancer progression and metastasis via an immune-dependent mechanisms. TAMs are commonly known for promoting tumor progression, angiogenesis, and immunosuppression, and targeting the activity of TAMs is an effective immunotherapy strategy [45]. Hypoxic areas of dying melanoma cells, release ADP as a locating signal

for P2Y12+ and move to the necrotic tumor environment and modulate the immune environment [45]. The study concluded that P2Y12 is a macrophage receptor which migrates to ADP-rich tumor areas, and modulates the immune response. Thus, while the role of P2Y12 on platelets has been a primary focus, it is clear that P2Y12 could also contribute to metastasis via other cell types as well. More work will be needed to fully decipher the cell-type specific contributions of P2Y12 to cancer metastasis.

Concerns have been raised about the relationship between P2Y12 antiplatelet treatment and tumor development or metastatic spread [48]. An ongoing populace-based study compared the risk of developing cancer between patients treated with solely aspirin or aspirin with clopidogrel. The study revealed that there was not a higher risk for cancer subjects that received combination treatment when compared to aspirin alone (HR 0.92, 95% CI: 0.86–0.97) and suggested that clopidogrel could decrease cancer occurrence [49]. The Food and Drug Administration (FDA) completed a meta-investigation to evaluate the impact of clopidogrel on death rates revealing that treatment with aspirin and clopidogrel was tolerated and effective for a year or more, showing no increased death rates [49].

When looking at the medication ticagrelor, there is more supporting evidence of a connection between metastasis and P2Y12 receptor inhibition in pancreatic malignancy. A recent study revealed that the P2Y12 receptor is expressed in pancreatic ductal adenocarcinoma (PDAC) cells and was required for malignant growth [50]. P2Y12

blockade with ticagrelor ended malignant cell development and synergized with chemotherapeutic drugs to reduce cell growth. The combination of ticagrelor and gemcitabine reduced tumor growth in both xenograft and syngeneic tumor mouse models tested. Although ticagrelor use increases bleeding risk by reducing the incidence of thrombotic events. P2Y12 contributed to pancreatic cancer cell survival and proliferation by advancing EGFR- and AKT-dependent survival signaling. This study showed that blocking P2Y12 with the clinically available antagonist ticagrelor reduced cancer cell proliferation, initiated apoptosis, and synergized with chemotherapeutics to robustly reduce cancer cell growth [50]. Combining ticagrelor and gemcitabine together reduced tumor development in both wild-type and immunocompromised xenograft mouse models, illustrating that ticagrelor might be an effective PDAC treatment, and potentially useful for other cancers with similar pathologies and underlying disease mechanisms [48].

PAR Antagonists

Prothrombin produces thrombin, a serine protease, after being cleaved during coagulation. Thrombin converts fibrinogen to fibrin, making it important to the coagulation cascade, and it is the strongest platelet agonist, making it vital to in the atherothrombotic process. The response to thrombin in platelets is mediated by GCPRs known as PARs located on the surface of the platelet. When the N terminal domain of prothrombin is cleaved by prothrombinase, PARs are activated by binding to the cleaved thrombin. The N-terminus that was cleaved then becomes an activation ligand that interacts with loop 2 domain and activates PAR signaling. There are four PARs that have been identified: PAR-1, PAR-2, PAR-3, and PAR-4. There is a high affinity for PAR-1

and low affinity for PAR-4 in human platelets, with no expression of PAR-2 and PAR-3, which are expressed in immune cells. PAR-1 was first identified in 1991 in a study of GPCRs [51], and is expressed in platelets, epithelial cells, and some immune cells [52]. PAR-1 is activated when thrombin binds with a specific sequence (LDPR41-S42) in the N-terminal site of the extracellular PAR-1 domain and cleaves the R41-S42 peptide bond. The unmasked sequence then binds intramolecularly, inducing signal transduction resulting in platelet activation [53]. Compared to PAR-1, PAR-4 lacks the negatively charged N-terminal sequence that binds to thrombin [54]. Therefore, a higher concentration of thrombin is needed for PAR-4 activation. Thrombin-induced cleavage of PAR-4 causes longer activation of Gαq leading to sustained release of Ca^{2+}, resulting in prolonged secondary signaling in the late-phase of platelet aggregation [54].

PARs have been established in their usage for vascular biology, hemostasis, and atherothrombosis, as receptors for thrombin [55]. The most widely used PAR inhibitor is Vorapaxar, and it is an antiplatelet agent that selectively inhibits thrombin actions through antagonism of PAR-1 [55]. Vorapaxar is a PAR-1 agonist used in a secondary preventative manner with prior myocardial infarction, but no history of stroke.

Some recent studies suggest that PARs could be used as potential therapeutic targets in cancer metastasis, alone or in combination with chemotherapy, antiangiogenic agents, and cytotoxic drugs. By looking at both *in vitro* and *in vivo* evidence, they showed that inhibiting PARs diminished tumor development, invasion and metastasis [56, 57, 58, 59].

There are two types of antagonists of the PAR receptors: functional receptors, which inhibit proteases, and pharmacological receptors, which inhibit tethered ligands or cleavage site of PAR [60, 61]. There could be clinical success through inhibiting PAR-1 on tumor cells. While looking at animal models, they discovered that thrombin-mediated PAR-1 and PAR-2 activation enhances the side effects of radiotherapy, like enteritis, where PAR-dependent signaling activates inflammatory, mitogenic, and proliferative processes in cells of the gastrointestinal tract after radiotherapy. The PAR-1 inhibitors reduced early, acute symptoms like enteritis but did not affect late-onset reactions [62, 63]. Other signaling pathways including αvβ5, EGFR, Erb, Erk, and MEK were induced when PAR was inhibited. These alternate pathways have the potential to develop into combination therapies to increase the effectiveness of PAR inhibitors in cancer.

PAR-1 overexpression has been observed in a variety of malignancies including melanoma, lung, breast, ovarian, and gastric cancer. The expression level of PAR-1 correlates with poor prognosis in the majority of these tumors [52]. In healthy cells, PAR-1 is tightly regulated unlike cancer cells where there is constant expression [64]. Thrombin activated PAR-1 induces EMT in gastric cancer cell lines [65]. Only in invasive breast cancer, thrombin-cleaved PAR-1 caused sustained ErbB/Her2 and EGFR activation [53]. In renal cancer, a PAR-1 variant was associated with an increased risk of metastasis and poor prognosis for patients [52]. Along with the data that PAR-1 induces EMT, in breast cancer cell line MCF-7, doxycycline, a member of tetracycline family with established anticancer activity inhibits EMT by inhibiting the PAR-1/NF-κB/miR-17/E-cadherin pathway [66]. Additionally, PAR-1 is activated by MMP-1 leading to Rho-

GTP and MAPK signaling cascade activation, which causes an increase in motility and cell proliferation. PAR-1 endows metastatic features to cancer cells by increasing the adhesiveness toward extracellular components. PAR-1 induction leads to assembly of focal adhesion contact (FAC) especially alpha(v)beta(5) integrin. Thus, antibody against alpha(v)beta(5) integrin works in disrupting PAR-1-dependent metastasis [67].

PAR-1 antagonists SCH79797 and RWJ56110 inhibited endothelial cell growth, tube formation in matrigel, and induced tumor cell apoptosis [68]. 4a-I-(2-cyclopropyl-3-ethynyl-4-(4-fluorophenyl) quinolone) ligands of PAR were synthesized, confirmed to block platelet aggregation, and reduced breast cancer cell growth via a PI3K-dependent mechanism [69]. In another article, it was found that the HPSE, an endo-β-D-glucuronidase that increases the bioavailability of pro-angiogenic mediators, and TRAP-6 protein both mediate an upregulation in the phosphorylation in focal adhesion kinase, both of which contribute to the metastatic niche formation. Both HPSE and TRAP-6 can be blocked by the PAR-1 inhibitor RWJ56110 used to target G-protein receptor signaling therefore reducing metastasis [70]. In another approach, PAR-1 small interfering RNA (siRNA) was incorporated into liposomes and assessed in experimental melanoma models. There was a decline in tumor growth, weight, and number of lung metastatic foci observed in mice treated with PAR-1 siRNA [71]. Angiogenesis was also affected by reducing VEGF, IL-8, and MM2 on MMP2 expression. PAR-1 silencing downregulates the expression of the adhesive protein MUC-18, which in turn reduces the invasiveness of melanoma cells [71].

To date, clinical testing of PAR inhibitors has been limited to diseases other than cancer. PAR-1 antagonists vorapaxar and atopaxar, for example, have been investigated in clinical trials in patients with acute coronary syndrome, cerebral infarction, and atherosclerosis [72, 73]. A better understanding of the clinical profile of this drug class will come from testing the specific impacts of PAR inhibitors in cancer metastasis. Yang et al. in 2009 studied the effects of blocking the PAR-1 pathway in breast cancer cells, from their results they concluded that blocking the pathway would be beneficial for the treatment of metastatic breast cancer [74]. Though much remains to be learned from clinical studies, preclinical results from studies inhibiting PARs to reduce cancer metastasis are promising and warrant further investigation of this drug class for suppression of cancer metastasis.

Antiplatelet drugs have been used to treat many types of CVD, and they are now beginning to play a key role in cancer patient treatment. As previously discussed, platelets are vital in the metastatic process including tumor survival and extravasation. With this knowledge, antiplatelet drugs are being analyzed for repurpose preclinically or clinically to treat or reverse cancer. This would allow a more customized approach to each patient's treatment. Results from drug repurposing studies targeting COX, TBXA2R, P2Y12, and PARs are summarized in **Table 1**.

Pathway	Potential Drugs	Cancers
COX-1	Aspirin	Breast Colorectal Prostate
Thromboxane	Ifetroban Linotroban Ramatroban Seratroban Sultroban Terutroban	Bladder Brain Breast Colorectal Lung Prostate
P2Y12	Cangrelor Clopidogel Elinogel Prasugel Ticagrelor	Breast Colorectal Lung Ovarian Prostate
Thrombin	Atopaxar Vlorapaxar	Breast Colorectal Pancreatic Prostate

Table 1 Four major platelet-activating pathways that promote metastasis

Results

When evaluating the types of cells that can drive malignancy, the following have been identified and put into a categorical cancer type: epithelial cell, connective tissue, blood-forming cells, and immune system cells where carcinomas, sarcoma, leukemia, and lymphoma, respectively, being the type of cancer they fall under [75]. Although antiplatelet drugs are effective at preventing the pathways mentioned in platelets, they have also been observed to block these pathways in other cell types. Arterial endothelial cells repair after an injury in rats were disrupted while being administered heparin, a thrombin inhibitor, indicating the impact of heparin on the repair mechanism [76]. In the same study, there was an observed defect in the smooth muscle repair of baboons while being administered heparin [76]. More intricate investigations into the cell-type dependence of malignancy caused via COX, TBXA2R, P2Y12, and PAR signaling will be essential toward improving our understanding of the contribution of these molecules to cancer metastasis.

Pharmacogenetics is the practice of analyzing a patient's genetic code to determine a personalized course of treatment for them. There are often analyses before and after treatment to evaluate the effectiveness of the treatment. Although this is not a widespread practice, it has the opportunity to create impact in the treatment of cancer. Nagasubramanian et al. analyzed the effectiveness of pharmacogenetics in 2003 by

looking at the polymorphisms in UGT1A1 and TPMT for different cancers. They found success in treating the cancers based on the genetic variations they found [77]. By identifying mutations, the results could reveal a mutation to one of the four drivers of malignancy discussed here, making treatment specialized to a patient's 'mutanome'. For instance, when probing the METABRIC breast cancer dataset in cBioPortal, there was a direct correlation between *TBXA2R* gene mutations and overall survival in 2170 patient cases, where *TBXA2R* mutation correlates with decreased survival (**Figure 3)**. From this information, we can conclude that using drugs that target this pathway, such as ramatroban and ifetroban, could benefit a large number of breast cancer patients and would be best suited to those who have hyperactivating mutations of the *TBXA2R* gene. Similarly, CRC has a high mutation rate for the COX2 enzyme and could benefit from treatment with aspirin (**Figure 4**). Indeed, the high mutation rate of COX2 in CRC could contribute to the effectiveness of low-dose aspirin at reducing CRC incidence in large cohorts of patients receiving aspirin daily. The study conducted by Chan et al. (2007) concluded that patients with overexpression of COX2 found a reduced risk of metastasis with regular low-dose aspirin usage [78]. However, there was a low sample number, 220 patient cases, on cBioPortal, therefore the survival curve did not indicate a significant difference in survival for patients with COX2 mutations but different results could be observed with a larger sample size. But the samples evaluated in this data set were any mutation, over or under expression, but the study by Chan et al. (2007) was specific to overexpression.

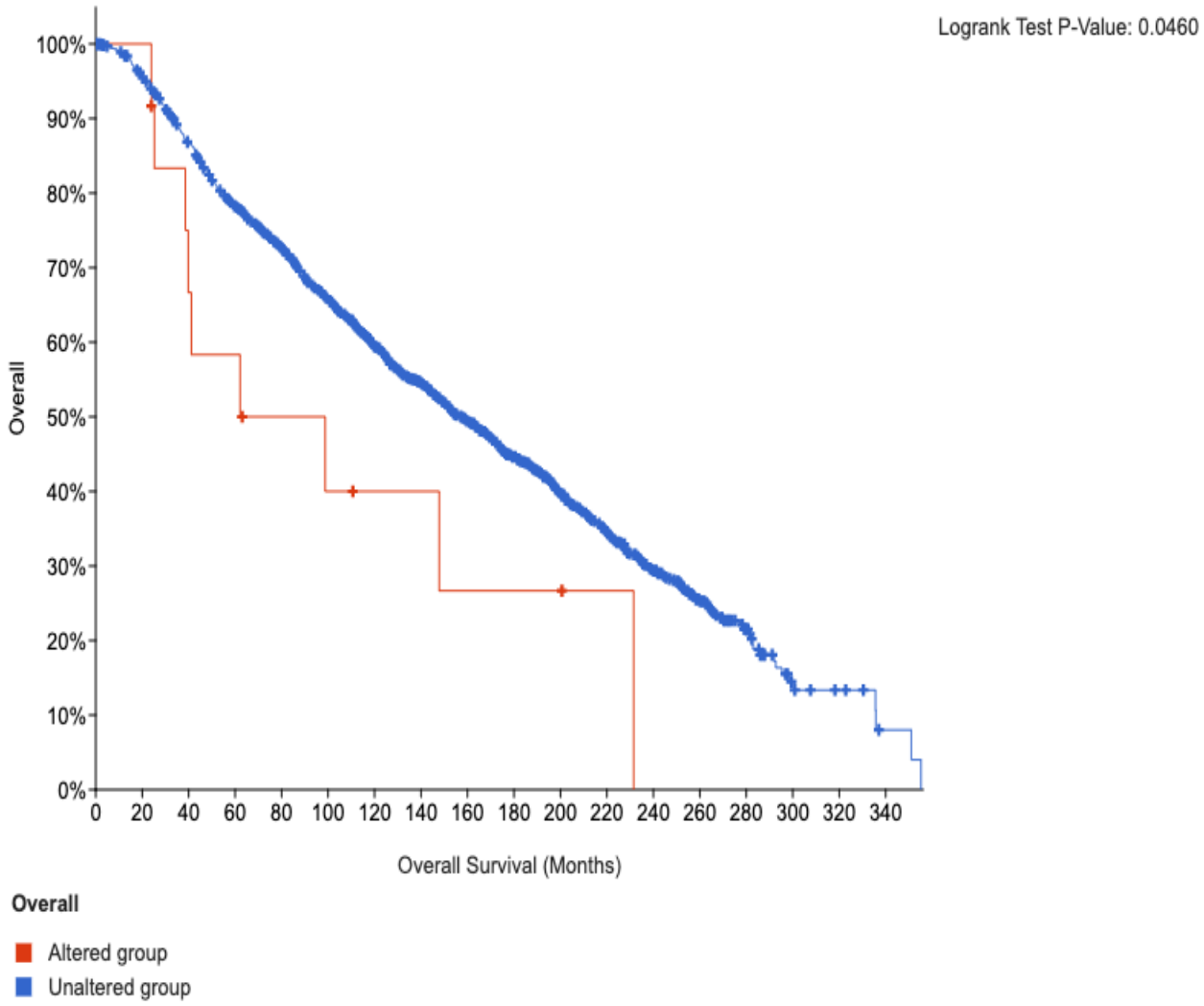

Figure 3 Survival Curve of Patients with METABRIC Breast Cancer: A comparison of patients' survival rates for those without mutations to *TBXA2R* gene (blue line) and those with mutation to the *TBXA2R* gene (red line).

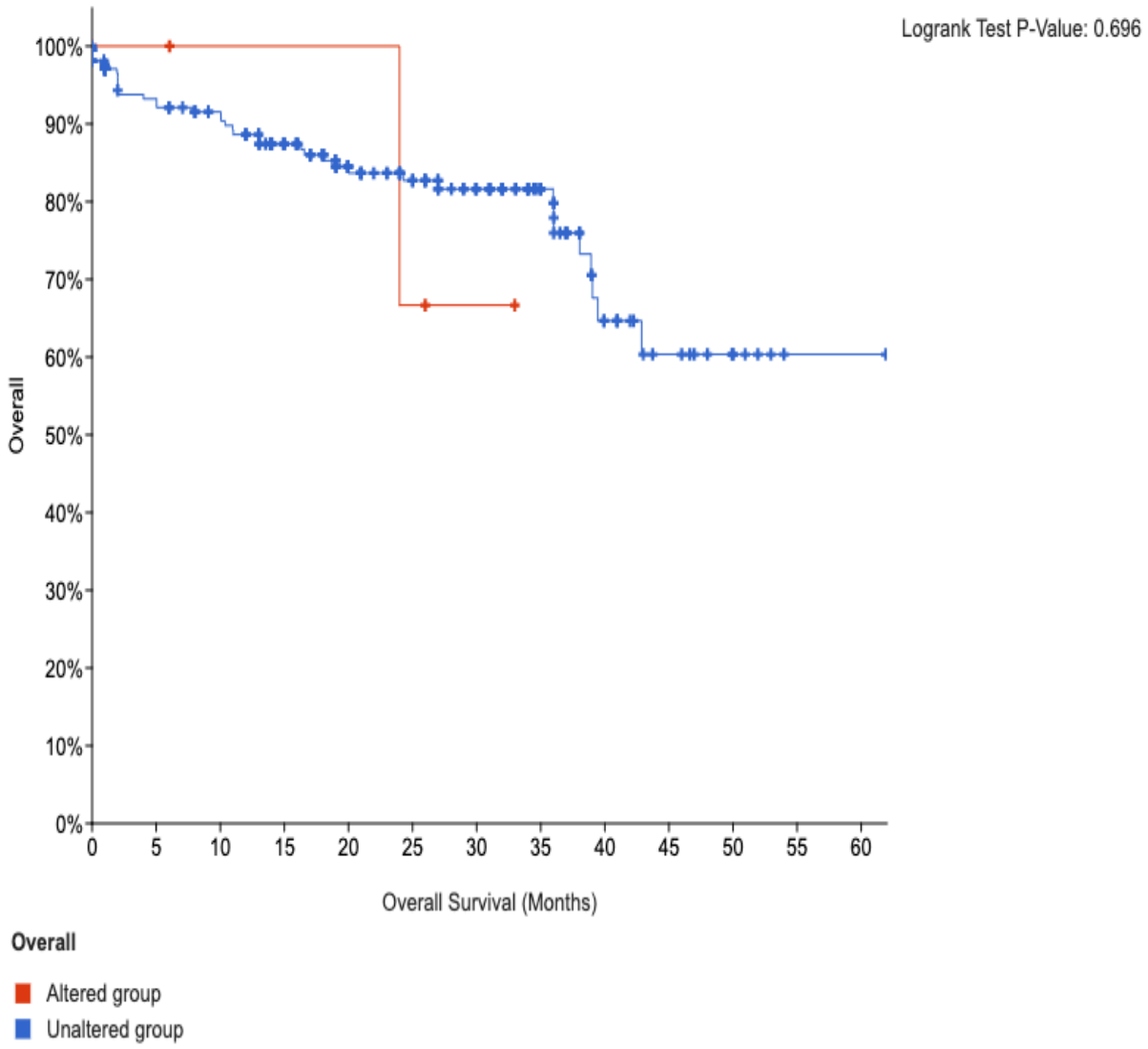

Figure 4 Survival Curve of Patients with TCGA, Firehouse Legacy Colorectal Cancer: A comparison of patients' survival rates for those without mutations to COX2 gene (blue line) and those with mutation to the COX2 gene (red line).

Aspirin, along with the other drugs listed previously in **Table 1** have been used to treat CVD, for decades because of their success and relatively low side effects. Aspirin is low

cost to patients and has shown low toxicity to their bodies. The greatest risk to patients is doubled the risk of gastric bleeding and an incidence of cerebral bleeding in fewer than 0.1% of patients [79]. Current trials are being conducted to continue to evaluate the effectiveness of aspirin in treating various types of cancer including colorectal [80, 81], breast [82, 83, 84], and prostate [85]. Another set of trials include Ifetroban, a thromboxane receptor antagonist, with results indicating that there is a hyperactivation of platelets and increased levels of thromboxane A2 [86]. Although there are many trials over the treatment of different cancer treatments, there needs to be further study of individualized treatment approaches for the pathways presented here.

Conclusion

Cancer metastasis is linked to platelet aggregation due to the platelets attaching to cancer cells and promoting a metastatic niche. There are four pathways that have been shown to robustly activate platelets: COX-1, thromboxane, P2Y12, thrombin. Each has a set of anticoagulant drugs that effectively block the pathway and have been well established for treating cardiovascular disease. Recent studies highlight a potential new role for these drugs in the prevention of platelet-mediated cancer metastasis and are reviewed here. The impact of antiplatelet drugs on cancer metastasis is driving the repurposing of these drugs as potentially transformative new agents in oncology. As repurposing is pursued, cell type-dependent effects of each agent, patient pharmacogenetics, and safety profiles should be carefully considered. These considerations will help determine which antiplatelet drug is best-suited to each patient and inform a personalized medicine approach for preventing metastasis with these agents. In sum, a broad pharmacopeia of safe and effective inhibitors of platelet activation exist and can be rapidly repurposed to prevent cancer metastasis. Clinical success of these agents would add an important and unique arrow to the oncologist's quiver because of their high tolerability and ability to directly inhibit metastasis.

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