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Antiplatelet Therapy Combined with Anastrozole Induces Features of Partial EMT in Breast Cancer Cells and Fails to Mitigate Breast-Cancer Induced Hypercoagulation

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Abstract: Thromboembolic complications are a leading cause of morbidity and mortality in cancer patients. Cancer patients often present with an increased risk for thrombosis including hypercoagulation, so the application of antiplatelet strategies to oncology warrants further investigation. This study investigated the effects of anastrozole and antiplatelet therapy (aspirin/clopidogrel cocktail or atopaxar) treatment on the tumour responses of luminal phenotype breast cancer cells and induced hypercoagulation. Ethical clearance was obtained (M150263). Blood was co-cultured with breast cancer cell lines (MCF7 and T47D) pre-treated with anastrozole and/or antiplatelet drugs for 24 h. Hypercoagulation was indicated by thrombin production and platelet activation (morphological and molecular). Gene expression associated with the epithelial-to-mesenchymal transition (EMT) was assessed in breast cancer cells, and secreted cytokines associated with tumour progression were evaluated. Data were analysed with the PAST3 software. Our findings showed that antiplatelet therapies (aspirin/clopidogrel cocktail and atopaxar) combined with anastrozole failed to prevent hypercoagulation and induced evidence of a partial EMT. Differences in tumour responses that modulate tumour aggression were noted between breast cancer cell lines, and this may be an important consideration in the clinical management of subphenotypes of luminal phenotype breast cancer. Further investigation is needed before this treatment modality (combined hormone and antiplatelet therapy) can be considered for managing tumour associated-thromboembolic disorder.

Keywords: breast cancer; hypercoagulation; aspirin; clopidogrel; atopaxar; anastrozole; platelet activation; thrombin activity; tumour survival

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1. Introduction

The relationship between tumour cells and platelets is complex, reciprocal, and implicated in driving hypercoagulation as a factor of tumorigenesis. Cancer patients typically present with thrombocytosis and features associated with hypercoagulation, and they are thus are at risk for thromboembolic complications, a leading cause of morbidity and mortality [1,2]. In post-menopausal patients presenting with hormone-dependent breast cancer, anastrozole (a non-steroidal aromatase inhibitor) is typically a first line treatment; however, while this drug improves survival, it is associated with thrombotic risk [3–5]. The addition of antiplatelet therapy to the management of cancer has been suggested; however, there remains a dearth of information on the efficacy of combined antiplatelet and hormone therapy on hypercoagulation and reciprocal effects on cancer.

The pathophysiology of thrombosis is underpinned by the dysregulation of the coagulation cascade and activities of cellular constituents including endothelial cells and platelets. Platelets are anucleate derivatives of megakaryocytes, approximately 2–3 µm in diameter with an average lifespan of seven days within the circulation [6]. Platelets present with a range of bioactive molecules including various cytokines, chemokines, and

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References

1. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Ca Cancer J. Clin.* **2018**, *68*, 394–424. [CrossRef]
2. Donnellan, E.; Khorana, A.A. Cancer and venous thromboembolic disease: A review. *Oncologist* **2017**, *22*, 199–207. [CrossRef] [PubMed]
3. Haddad, T.C.; Greeno, E.W. Chemotherapy-induced thrombosis. *Thromb. Res.* **2006**, *118*, 555–568. [CrossRef] [PubMed]
4. Cuzick, J.; Sestak, I.; Forbes, J.F.; Dowsett, M.; Knox, J.; Cawthorn, S.; Saunders, C.; Roche, N.; Mansel, R.E.; Minckwitz, G.; et al. Anastrozole for prevention of breast cancer in high-risk postmenopausal women (IBIS-II): An international, double-blind, randomised placebo-controlled trial. *Lancet* **2014**, *383*, 1041–1048. [CrossRef]
5. Kemp, A.; Preen, D.B.; Saunders, C.; Boyle, F.; Bulsara, M.; Holman, C.D.J.; Malacova, E.; Roughead, E.E. Women commencing anastrozole, letrozole or tamoxifen for early breast cancer: The impact of comorbidity and demographics on initial choice. *PLoS ONE* **2014**, *9*, e84835. [CrossRef]
6. Haemmerle, M.; Stone, R.L.; Menter, D.G.; Afshar-Kharghan, V.; Sood, A.K. The Platelet Lifeline to Cancer: Challenges and Opportunities. *Cancer Cell* **2018**, *33*, 965–983. [CrossRef] [PubMed]
7. Thon, J.N.; Italiano, J.E. Platelets: Past, present and future. *Handb. Exps. Pharm.* **2012**, *210*, 4–20. [CrossRef]
8. Owens, A.P.; MacKman, N. Microparticles in hemostasis and thrombosis. *Circ. Res.* **2011**, *108*, 1284–1297. [CrossRef] [PubMed]
9. Van der Pol, E.; Böing, A.N.; Harrison, P.; Sturk, A.; Nieuwland, R. Classification, functions, and clinical relevance of extracellular vesicles. *Pharm. Rev.* **2012**, *64*, 676–705. [CrossRef]
10. Menter, D.G.; Tucker, S.C.; Kopetz, S.; Sood, A.K.; Crissman, J.D.; Honn, K.V. Platelets and cancer: A casual or causal relationship: Revisited. *Cancer Metastasis Rev.* **2014**, *33*, 231–269. [CrossRef]
11. Phillips, K.G.; Lee, A.M.; Tormoen, G.W.; Rigg, R.A.; Kolatkar, A.; Luttgen, M.; Bethel, K.; Bazhenova, L.; Kuhn, P.; Newton, P.; et al. The thrombotic potential of circulating tumor microemboli: Computational modeling of circulating tumor cell-induced coagulation. *Am. J. Physiol. Cell Physiol.* **2015**, *308*, C229–C236. [CrossRef] [PubMed]
12. Date, K.; Hall, J.; Greenman, J.; Maraveyas, A.; Madden, L.A. Tumour and microparticle tissue factor expression and cancer thrombosis. *Thromb. Res.* **2013**, *131*, 109–115. [CrossRef]
13. Orellana, R.; Kato, S.; Erices, R.; Bravo, M.L.; Gonzalez, P.; Oliva, B.; Cubillos, S.; Valdivia, A.; Ibañez, C.; Brañes, J.; et al. Platelets enhance tissue factor protein and metastasis initiating cell markers, and act as chemoattractants increasing the migration of ovarian cancer cells. *BMC Cancer* **2015**, *15*, 290. [CrossRef] [PubMed]
14. Li, Z.; Dong, M.; Fan, D.; Hou, P.; Li, H.; Liu, L.; Lin, C.; Liu, J.; Su, L.; Wu, L.; et al. LncRNA ANCR down-regulation promotes TGF-β-induced EMT and metastasis in breast cancer. *Oncotarget* **2017**, *8*, 67329–67343. [CrossRef]
15. Aschermann, M. Prevention of arterial and venous thrombosis in cancer patients. *Cor Vasa* **2013**, *55*, e196–e200. [CrossRef]
16. Roop, R.P.; Naughton, M.J.; Van Poznak, C.; Schneider, J.G.; Lammers, P.E.; Pluard, T.J.; Johnson, F.; Eby, C.S.; Weilbaecher, K.N. A randomized phase II trial investigating the effect of platelet function inhibition on circulating tumor cells in patients with metastatic breast cancer. *Clin. Breast Cancer* **2013**, *13*, 409–415. [CrossRef] [PubMed]
17. Charrin, S.; Jouannet, S.; Boucheix, C.; Rubinstein, E. Tetraspanins at a glance. *J. Cell. Sci.* **2014**, *127*, 3641–3648. [CrossRef] [PubMed]
18. Seubert, B.; Cui, H.; Simonavicius, N.; Honert, K.; Schäfer, S.; Reuning, U.; Heikenwalder, M.; Mari, B.; Krüger, A. Tetraspanin CD63 acts as a pro-metastatic factor via β-catenin stabilization. *Int. J. Cancer* **2015**, *136*, 2304–2315. [CrossRef] [PubMed]
19. Yan, M.J.; Jurasz, P. The role of platelets in the tumor microenvironment: From solid tumors to leukemia. *Biochim. Biophys. Acta Mol. Cell. Res.* **2016**, *1863*, 392–400. [CrossRef] [PubMed]
20. Zhang, Q.; Liu, H.; Zhu, Q.; Zhan, P.; Zhu, S.; Zhang, J.; Lv, T.; Song, Y. Patterns and functional implications of platelets upon tumor "education". *Int. J. Biochem. Cell. Biol.* **2017**, *90*, 68–80. [CrossRef]
21. Plantureux, L.; Crescence, L.; Dignat-George, F.; Panicot-Dubois, L.; Dubois, C. Effects of platelets on cancer progression. *Thromb. Res.* **2018**, *164*, S40–S47. [CrossRef]
22. Li, N. Platelets in cancer metastasis: To help the "villain" to do evil. *Int. J. Cancer* **2016**, *138*, 2078–2087. [CrossRef] [PubMed]
23. Moschonas, I.C.; Goudevenos, J.A.; Tselepis, A.D. Protease-activated receptor-1 antagonists in long-term antiplatelet therapy. Current state of evidence and future perspectives. *Int. J. Cardiol.* **2015**, *185*, 9–18. [CrossRef] [PubMed]

24. Capodanno, D.; Ferreiro, J.L.; Angiolillo, D.J. Antiplatelet therapy: New pharmacological agents and changing paradigms. *J. Thrombs Haemost.* **2013**, *11*, 316–329. [CrossRef]
25. Leader, A.; Zelikson-Saporta, R.; Pereg, D.; Spectre, G.; Rozovski, U.; Raanani, P.; Hermoni, D.; Lishner, M. The Effect of Combined Aspirin and Clopidogrel Treatment on Cancer Incidence. *Am. J. Med.* **2017**, *130*, 826–832. [CrossRef] [PubMed]
26. Tesfamariam, B. Involvement of platelets in tumor cell metastasis. *Pharm. Ther.* **2016**, *157*, 112–119. [CrossRef]
27. Liu, B.; Qu, L.; Yan, S. Cyclooxygenase-2 promotes tumor growth and suppresses tumor immunity. *Cancer Cell. Int.* **2015**, *15*, 2–7. [CrossRef]
28. Kochel, T.J.; Goloubeva, O.G.; Fulton, A.M. Upregulation of cyclooxygenase-2/prostaglandin E2 (COX-2/PGE2) pathway member multiple drug resistance-associated protein 4 (MRP4) and downregulation of prostaglandin transporter (PGT) and 15-prostaglandin dehydrogenase (15-PGDH) in triple-negative breast. *Breast Cancer Basic Clin. Res.* **2016**, *10*, 61–70. [CrossRef]
29. Ballerini, P.; Dovizio, M.; Bruno, A.; Tacconelli, S.; Patrignani, P. P2Y 12 Receptors in Tumorigenesis and Metastasis. *Front. Pharm.* **2018**, *9*, 1–8. [CrossRef]
30. Even-Ram, S.; Uziely, B.; Cohen, P.; Grisaru-Granovsky, S.; Maoz, M.; Ginzburg, Y.; Reich, R.; Vlodavsky, I.; Bar-Shavit, R. Thrombin receptor overexpression in malignant and physiological invasion processes. *Nat. Med.* **1998**, *4*, 909–914. [CrossRef]
31. Hoellen, F.; Kelling, K.; Dittmer, C.; Diedrich, K.; Friedrich, M.; Thill, M. Impact of cyclooxygenase-2 in breast cancer. *Anticancer Res.* **2011**, *31*, 4359–4367. [PubMed]
32. Cattaneo, M.; Schulz, R.; Nylander, S. Adenosine-Mediated Effects of Ticagrelor Evidence and Potential Clinical Relevance. *J. Am. Coll. Cardiol.* **2014**, *63*, 2503–2509. [CrossRef] [PubMed]
33. Hechler, B.; Gachet, C. Purinergic Receptors in Thrombosis and Inflammation. *Arter. Thromb. Vasc. Biol.* **2015**, *35*, 2307–2315. [CrossRef] [PubMed]
34. Wang, Y.; Liu, J.; Ying, X.; Lin, P.C.; Zhou, B.P. Twist-mediated Epithelial-mesenchymal Transition Promotes Breast Tumor Cell Invasion via Inhibition of Hippo Pathway. *Sci. Rep.* **2016**, *6*, 1–10. [CrossRef] [PubMed]
35. McDonald, M.G.; Ray, S.; Amorosi, C.J.; Sitko, K.A.; Kowalski, J.P.; Paco, L.; Nath, A.; Gallis, B.; Totah, R.A.; Dunham, M.J.; et al. Expression and functional characterization of breast cancer-associated cytochrome P450 4Z1 in saccharomyces cerevisiae. *Drug Metab. Dispos* **2017**, *45*, 1364–1371. [CrossRef] [PubMed]
36. Elaskalani, O.; Berndt, M.C.; Falasca, M.; Metharom, P. Targeting platelets for the treatment of cancer. *Cancers* **2017**, *9*, 94. [CrossRef]
37. Gresele, P.; Malvestiti, M.; Momi, S. Anti-platelet treatments in cancer: Basic and clinical research. *Thromb. Res.* **2018**, *164*, 106–111. [CrossRef]
38. de Leite, A.M.; Macedo, A.V.S.; Jorge, A.J.L.; Martins, W.A. Antiplatelet therapy in breast cancer patients using hormonal therapy: Myths, evidence and potentialities—Systematic review. *Arq. Bras. Cardiol.* **2018**, *111*, 205–212. [CrossRef]
39. Roe, M.T.; Cyr, D.D.; Eckart, D.; Schulte, P.J.; Morse, M.A.; Blackwell, K.L.; Ready, N.E.; Zafar, S.Y.; Beaven, A.W.; Strickler, J.H.; et al. Ascertainment, classification, and impact of neoplasm detection during prolonged treatment with dual antiplatelet therapy with prasugrel vs. clopidogrel following acute coronary syndrome. *Eur. Heart J.* **2016**, *37*, 412–422. [CrossRef]
40. Mauri, L.; Elmariah, S.; Yeh, R.W.; Cutlip, D.E.; Steg, P.G.; Windecker, S.; Wiviott, S.D.; Cohen, D.J.; Massaro, J.M.; D'Agostino, R.B., Sr.; et al. Causes of late mortality with dual antiplatelet therapy after coronary stents. *Eur. Heart J.* **2016**, *37*, 378–385. [CrossRef] [PubMed]
41. Smeda, M.; Kieronska, A.; Proniewski, B.; Jasztal, A.; Selmi, A.; Wandzel, K.; Zakrzewska, A.; Wojcik, T.; Przyborowski, K.; Derszniak, K. Dual antiplatelet therapy with clopidogrel and aspirin increases mortality in 4T1 metastatic breast cancer-bearing mice by inducing vascular mimicry in primary tumour. *Oncotarget* **2018**, *9*, 17810–17824. [CrossRef] [PubMed]
42. Bhatt, D.L.; Fox, K.A.A.; Hacke, W.; Berger, B.P.; Black, H.R.; Boden, W.E.; Cacoub, P.; Cohen, E.A.; Creager, M.A.; Easton, J.D.; et al. Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events. *N. Engl. J. Med.* **2006**, *354*, 1706–1717. [CrossRef] [PubMed]
43. Denslow, A.; Świtalska, M.; Jarosz, J.; Papiernik, D.; Porshneva, K.; Nowak, M.; Wietrzyk, J. Clopidogrel in a combined therapy with anticancer drugs—effect on tumor growth, metastasis, and treatment toxicity: Studies in animal models. *PLoS ONE* **2017**, *12*, e0188740. [CrossRef]
44. Sun, J.; Du, Y.; Zhang, X.; Wang, Z.; Lin, Y.; Song, Q.; Wang, X.; Guo, J.; Li, S.; Nan, J.; et al. Discovery and evaluation of Atopaxar hydrobromide, a novel JAK1 and JAK2 inhibitor, selectively induces apoptosis of cancer cells with constitutively activated STAT3. *Investig. New Drugs* **2019**. [CrossRef]
45. Tripodi, A. Thrombin generation assay and its application in the clinical laboratory. *Clin. Chem.* **2016**, *62*, 699–707. [CrossRef]
46. He, P.; Zhang, F.; Zhong, C.; Li, M.; Zheng, J.; Hua, B.; Sun, J. Timely and large dose of clotting factor IX provides better joint wound healing after hemarthrosis in hemophilia B mice. *Int. J. Hematol.* **2019**, *110*, 59–68. [CrossRef] [PubMed]
47. Xu, X.R.; Carrim, N.; Neves, M.A.D.; McKeown, T.; Stratton, T.W.; Coelho, R.M.P.; Lei, X.; Chen, P.; Xu, J.; Dai, X.; et al. Platelets and platelet adhesion molecules: Novel mechanisms of thrombosis and anti-thrombotic therapies. *Thromb. J.* **2016**, *14*, 37–46. [CrossRef]
48. Navred, K.; Martin, M.; Ekdahl, L.; Zetterberg, E.; Andersson, N.G.; Strandberg, K.; Norstrom, E. A simplified flow cytometric method for detection of inherited platelet disorders—A comparison to the gold standard light transmission aggregometry. *PLoS ONE* **2019**, *14*, 1–12. [CrossRef] [PubMed]

49. Baaten, C.C.F.M.J.; Ten Cate, H.; Van der Meijden, P.E.L.; Heemskerk, J.W.M. Platelet populations and priming in hematological diseases. *Blood Rev.* **2017**, *31*, 389–399. [CrossRef]
50. Bambace, N.M.; Holmes, C.E. The platelet contribution to cancer progression. *JTH* **2011**, *9*, 237–249. [CrossRef] [PubMed]
51. Labelle, M.; Begum, S.; Hynes, R.O. Direct Signaling between Platelets and Cancer Cells Induces an Epithelial-Mesenchymal-Like Transition and Promotes Metastasis. *Cancer Cell* **2011**, *20*, 576–590. [CrossRef] [PubMed]
52. Lal, I.; Dittus, K.; Holmes, C.E. Platelets, coagulation and fi brinolysis in breast cancer progression. *Breast Cancer Res.* **2013**, *15*, 207. [CrossRef] [PubMed]
53. Fuentes, E.; Palomo, I.; Rojas, A. Cross-talk between platelet and tumor microenvironment: Role of multiligand/RAGE axis in platelet activation. *Blood Rev.* **2016**, *30*, 213–221. [CrossRef] [PubMed]
54. Leblanc, R.; Peyruchaud, O. Metastasis: New functional implications of platelets and megakaryocytes. *Blood* **2016**, *128*, 24–31. [CrossRef]
55. Bruno, A.; Dovizio, M.; Tacconelli, S.; Contursi, A.; Ballerini, P.; Patrignami, P. Antithrombotic agents and cancer. *Cancers* **2018**, *10*, 253. [CrossRef]
56. Elmariah, S.; Doros, G.; Benavente, O.R.; Bhatt, D.L.; Connolly, S.J.; Yusuf, S.; Steinhubl, S.R.; Liu, Y.; Hsieh, W.H.; Yeh, R.W.; et al. Impact of Clopidogrel Therapy on Mortality and Cancer in Patients with Cardiovascular and Cerebrovascular Disease: A Patient-Level Meta-Analysis. *Circ. Cardiovasc. Interv.* **2018**, *11*, e005795. [CrossRef]
57. Herkert, O.; Kuhl, H.; Sandow, J.; Busse, R.; Schini-Kerth, V.B. Sex steroids used in hormonal treatment increase vascular procoagulant activity by inducing thrombin receptor (PAR-1) expression. *Circulation* **2001**, *104*, 2826–2831. [CrossRef]
58. Nag, J.; Bar-Shavit, R. Transcriptional landscape of PARs in epithelial malignancies. *Int. J. Mol. Sci.* **2018**, *19*, 3451. [CrossRef]
59. Jick, H.; Kaye, J.A.; Vasilakis-Scaramozza, C.; Jick, S.S. Risk of venous thromboembolism among users of third generation oral contraceptives compared with users of oral contraceptives with levonorgestrel before and after 1995: Cohort and case–control analysis. *BMJ* **2000**, *321*, 1190–1195. [CrossRef]
60. Van dey Spuy, W.J.; Augustine, T.N. Ultrastructural investigation of the time-dependent relationship between breast cancer cells and thrombosis induction. *Micron* **2016**, *90*, 59–63. [CrossRef]
61. Pather, K.; Dix-Peek, T.; Duarte, R.; Chetty, N.; Augustine, T.N. Breast cancer cell-induced platelet activation is compounded by tamoxifen and anastrozole in vitro. *Thromb. Res.* **2019**, *177*, 51–58. [CrossRef]
62. Pather, K.; Augustine, T. Tamoxifen induces hypercoagulation and alterations in Erα and ERβ dependent on breast cancer sub-phenotype ex vivo. *Sci. Rep.* **2020**, *10*, 19256. [CrossRef]
63. Stalker, T.J.; Newman, D.K.; Ma, P.; Wannemacher, K.M.; Brass, L.F. Platelet signaling. *Handb. Exp. Pharm.* **2012**, *210*, 59–85. [CrossRef]
64. Tomaiuolo, M.; Brass, L.F.; Stalker, T.J. Regulation of Platelet Activation and Coagulation and Its Role in Vascular Injury and Arterial Thrombosis. *Interv. Cardiol. Clin.* **2017**, *6*, 1–12. [CrossRef]
65. Augustine, T.N. The aegis: Platelets as biomarkers of tumor progression. *Biomark Med.* **2020**, *14*, 573–585. [CrossRef] [PubMed]
66. Swieringa, F.; Spronk, H.M.H.; Heemskerk, J.W.M.; Van der Meijden, P.E.J. Integrating platelet and coagulation activation in fibrin clot formation. *Res. Pr. Thromb. Haemost.* **2018**, *2*, 450–460. [CrossRef]
67. Augustine, T.N.; Van der Spuy, W.J.; Kaberry, L.L.; Shayi, M. Thrombin-Mediated Platelet Activation of Lysed Whole Blood and Platelet-Rich Plasma: A Comparison Between Platelet Activation Markers and Ultrastructural Alterations. *Microsc. Microanal.* **2016**, *22*, 630–639. [CrossRef] [PubMed]
68. Lazar, S.; Goldfinger, L.E. Platelet Microparticles and miRNA Transfer in Cancer Progression: Many Targets, Modes of Action, and Effects across Cancer Stages. *Front. Cardiovasc. Med.* **2018**, *5*, 1–6. [CrossRef]
69. Vences-catalán, F.; Levy, S. Immune Targeting of Tetraspanins Involved in Cell Invasion and Metastasis. *Front. Immunol.* **2018**, *9*, 1–7. [CrossRef] [PubMed]
70. Pols, M.S.; Klumperman, J. Trafficking and function of the tetraspanin CD63. *Exp. Cell Res.* **2009**, *315*, 1584–1592. [CrossRef] [PubMed]
71. Passacquale, G.; Ferro, A. Current concepts of platelet activation: Possibilities for therapeutic modulation of heterotypic vs. homotypic aggregation. *Bri. J. Clin. Pharm.* **2011**, *72*, 604–618. [CrossRef]
72. Miyashita, T.; Tajima, H.; Makino, I.; Nakagawara, H.; Kitagawa, H.; Fushida, S.; Harmon, J.W.; Ohta, T. Metastasis-promoting role of extravasated platelet activation in tumor. *J. Surg Res.* **2015**, *193*, 289–294. [CrossRef]
73. Pang, J.H.; Coupland, L.A.; Freeman, C.; Chong, B.H.; Parish, C.R. Activation of tumour cell ECM degradation by thrombin-activated platelet membranes: Potentially a P-selectin and GPIIb/IIIa-dependent process. *Clin. Exp. Metastasis* **2015**, *32*, 495–505. [CrossRef] [PubMed]
74. Guillem-Llobat, P.; Dovizio, M.; Bruno, A.; Ricciotti, E.; Cufino, V.; Sacco, A.; Grande, R.; Alberti, S.; Arena, V.; Cirillo, M.; et al. Aspirin prevents colorectal cancer metastasis in mice by splitting the crosstalk between platelets and tumor cells. *Oncotarget* **2016**, *7*, 32462–32477. [CrossRef] [PubMed]
75. Hua, H.; Zhang, H.; Kong, Q.; Jiang, Y. Mechanisms for estrogen receptor expression in human cancer. *Exp. Hematol. Oncol.* **2018**, *7*, 1–11. [CrossRef]
76. Mauri, L.; Kereiakes, D.J.; Yeh, R.W.; Driscoll-Shempp, P.; Cutlip, D.E.; Steg, G.; Normand, S.L.T.; Braunwald, E.; Wiviott, S.D.; Cohen, D.J.; et al. Twelve or 30 Months of Dual Antiplatelet Therapy After Drug-eluting Stents. *N. Engl. J. Med.* **2014**, *371*, 2155–2166. [CrossRef] [PubMed]

77. Xulu, K.; Duarte, R.; Augustine, T. Combined Anastrozole and Antiplatelet therapy treatment differentially promotes breast cancer cell survival. *Microsc. Microanal.* **2020**, *26*, 497–508. [CrossRef]
78. Araki, K.; Shimura, T.; Suzuki, H.; Tsutsumi, S.; Wada, W.; Yajima, T.; Kobayahi, T.; Kubo, N.; Kuwano, H. E/N-cadherin switch mediates cancer progression via TGF-β-induced epithelial-to-mesenchymal transition in extrahepatic cholangiocarcinoma. *Br. J. Cancer* **2011**, *105*, 1885–1893. [CrossRef] [PubMed]
79. Aleskandarany, M.A.; Negm, O.H.; Green, A.R.; Ahmed, M.A.H.; Nolan, C.C.; Tighe, P.J.; Ellis, I.O.; Rakha, E.A. Epithelial mesenchymal transition in early invasive breast cancer: An immunohistochemical and reverse phase protein array study. *Breast Cancer Res. Treat.* **2014**, *145*, 339–348. [CrossRef]
80. Jolly, M.K.; Boareto, M.; Huang, B.; Jia, D.; Lu, M.; Ben-Jacob, E.; Onuchic, J.N.; Levine, H. Implications of the hybrid epithelial/mesenchymal phenotype in metastasis. *Front. Oncol* **2015**, *5*, 155. [CrossRef]
81. Saitoh, M.J.B. Cellular plasticity in epithelial homeostasis and diseases: Involvement of partial EMT in cancer progression. *J. Biochem.* **2018**, *164*, 257–264. [CrossRef] [PubMed]
82. Covic, L.; Kuliopulos, A. Protease-activated receptor 1 as therapeutic target in breast, lung, and ovarian cancer: Pepducin approach. *Int. J. Mol. Sci.* **2018**, *19*, 2237. [CrossRef] [PubMed]
83. O'Donoghue, M.; Bhatt, D.L.; Wiviott, S.D.; Goodman, S.G.; Fitzgerald, D.J.; Angiolillo, D.J.; Goto, S.; Montalescot, G.; Zeymer, U.; Aylward, P.E.; et al. Safety and Tolerability of Atopaxar in the Treatment of The Lessons From Antagonizing the Cellular Effects of Thrombin—Acute Coronary Syndromes Trial. *Circulation* **2011**, *123*, 1843–1853. [CrossRef] [PubMed]
84. Pan, H.; Boucher, M.; Kaunelis, D. PAR-1 Antagonists: An Emerging Antiplatelet Drug Class. *Emerg. Health Technol.* **2016**, *148*, 1–17.
85. Wojtukiewicz, M.Z.; Hempel, D.; Sierko, E.; Tucker, S.C.; Honn, K.V. Thrombin—Unique coagulation system protein with multifaceted impacts on cancer and metastasis. *Cancer Metastasis Rev.* **2016**, *35*, 213–233. [CrossRef]
86. Gomes, F.G.; Sandim, V.; Almeida, V.H.; Rondon, A.M.R.; Succar, B.B.; Hottz, E.D.; Leal, A.C.; Vercoza, B.R.F.; Rodrigues, J.C.F.; Bozza, P.T.; et al. Breast-cancer extracellular vesicles induce platelet activation and aggregation by tissue factor-independent and -dependent mechanisms. *Thromb. Res.* **2017**, *159*, 24–32. [CrossRef]
87. Kamath, L.; Meydani, A.; Foss, F. Signaling from Protease-activated Receptor-1 Inhibits Migration and Invasion of Breast Cancer Cells. *Cancer Res.* **2001**, *61*, 5933–5940.
88. Egan, K.; Crowley, D.; Smyth, P.; O'Toole, S.O.; Spillane, C.; Martin, C.; Gallagher, M.; Canney, A.; Norris, L.; Conlon, N.; et al. Platelet Adhesion and Degranulation Induce Pro-Survival and Pro-Angiogenic Signalling in Ovarian Cancer Cells. *PLoS ONE* **2011**, *6*, e26125. [CrossRef]
89. Liu, X.; Yu, J.; Song, S.; Yue, X.; Li, Q. Protease-activated receptor-1 (PAR-1): A promising molecular target for cancer. *Oncotarget* **2017**, *8*, 107334–107345. [CrossRef]
90. Kogushi, M.; Matsuola, T.; Kawata, T.; Kuramochi, H.; Kawaguchi, S.; Murakami, K.; Hiyoshi, H.; Suzuki, S.; Kawahar, T.; Kajiwara, A.; et al. The novel and orally active thrombin receptor antagonist E5555 (Atopaxar) inhibits arterial thrombosis without affecting bleeding time in guinea pigs. *Eur. J. Pharm.* **2011**, *657*, 131–137. [CrossRef]
91. Chatterjee, S.; Sharma, A.; Mukherjee, D. PAR-1 antagonists: Current state of evidence. *J. Thromb.* **2013**, *35*, 1–9. [CrossRef]
92. Yu, S.; Kim, T.; Yoo, K.H.; Kang, K. The T47D cell line is an ideal experimental model to elucidate the progesterone-specific effects of a luminal A subtype of breast cancer. *Biochem. Biophys. Res. Commun.* **2017**, *486*, 752–758. [CrossRef]
93. Dupuis, M.; Severin, S.; Noirrit-Esclassan, E.; Arnal, J.F.; Payrastre, B.; Valera, M.C. Effects of estrogens on platelets and megakaryocytes. *Int. J. Mol. Sci.* **2019**, *20*, 3111. [CrossRef]
94. Voelker, M.; Hammer, M. Dissolution and pharmacokinetics of a novel micronized aspirin formulation. *Inflammopharmacology* **2012**, *20*, 225–231. [CrossRef] [PubMed]
95. Zou, J.J.; Tan, J.; Fan, H.W.; Chen, S.L. Bioequivalence study of clopidogrel 75 Mg tablets in healthy male volunteers. *J. Bioequivalence Bioavailab.* **2012**, *4*, 6–9. [CrossRef]
96. Leytin, V.; Mody, M.; Semple, J.W.; Garvey, B.; Freedman, J. Flow Cytometric Parameters for Characterizing Platelet Activation by Measuring P-Selectin (CD62) Expression: Theoretical Consideration and Evaluation in Thrombin-Treated Platelet Populations. *Biochem. Biophys. Res. Commun.* **2000**, *269*, 85–90. [CrossRef]
97. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. Past: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **2001**, *4*, 1–9.
98. Wong, H.L.; Pfeiffer, R.M.; Fears, T.R.; Vermeulen, R.; Ji, S.; Rabkin, C.S. Reproducibility and correlations of multiplex cytokine levels in asymptomatic persons. *Cancer Epidemiol. Biomarkers Prev.* **2008**, *17*, 3450–3456. [CrossRef] [PubMed]
99. Ballenberger, N.; Lluis, A.; von Mutius, E.; Illi, S.; Schaub, B. Novel statistical approaches for non-normal censored immunological data: Analysis of cytokine and gene expression data. *PLoS ONE* **2012**, *7*, e46423. [CrossRef] [PubMed]
100. Augustine, T.N.; Duarte, R.; Candy, G.P. Breast cancer cells induce a pro-inflammatory response to mitigate immune mediation in a 3D culture model. *Anticancer Res.* **2020**, *40*, 6179–6193. [CrossRef]
101. Hellemans, J.; Mortier, G.; De Paepe, A.; Speleman, F.; Vandesompele, J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biol.* **2007**, *8*, R19. [CrossRef]
102. Goni, R.; García, P.; Foissac, S. The qPCR data statistical analysis. *Integromics SL* **2009**, *1*, 1–9.