Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

PAR4 Antagonists (BMS-986120, BMS-986141)

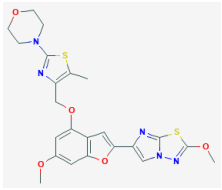
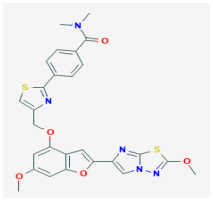
Evidence Summary

May offer a better therapeutic window and less bleeding risk than other anti-platelet therapies. May protect against thrombosis and pathogenic vascular inflammation.

Neuroprotective Benefit: PAR4 inhibition may mitigate thrombin-associated inflammation and neurovascular pathology.

Aging and related health concerns: PAR4 inhibition may protect against thrombosis and diabetes-related cardiovascular complications.

Safety: May increase risk for bleeding, but to a lesser degree than existing anti-platelet therapies. Additional immunosuppressant effects or clinical population dependent effects are possible.

<table>
<tr><td>Availability: Research use</td><td>Dose: Not established</td><td rowspan="2">BMS-986120
Chemical formula: $C_{23}H_{23}N_5O_5S_2$
MW: 513.6 g/mol
 
Source: PubChem
BMS-986141
Chemical formula: $C_{27}H_{23}N_5O_5S_2$
MW: 561.6 g/mol
 
Source: PubChem</td></tr>
<tr><td>Half-life:
BMS-986120
Plasma half-life ~ 4 hours
BMS-986141
Terminal half-life 35.2-45.5 hours</td><td>BBB: Not established</td></tr>
<tr><td>Clinical trials: BMS-986120 Phase 1 in healthy volunteers (n=66, n=40)
BMS-986141
Phase 1 in healthy volunteers
(n=148, n=16, n=16)
Phase 2 in stroke (n=16)
Clinical development discontinued.</td><td>Observational studies:
None for PAR4 antagonists.
SNP in PAR4 associated with higher expression on platelets is associated with increased risk for stroke.</td><td></td></tr>
</table>

What is it?

Protease-activated receptor (PAR)4 belongs to a class of G-protein coupled receptors (GPCRs) that are activated by serine proteases [1]. PARs play important roles in hemostasis, thrombosis, inflammation, and cell proliferation. Although thrombin is the best characterized activator, PAR4 is also activated by a variety of proteases released in response to cellular injury, such as cathepsin and trypsin. PAR4 is expressed on a variety of cell types, but aside from platelets, expression tends to be low under basal conditions, and upregulated in response to cell stressors [2]. Human platelets express PAR1 and PAR4, which together mediate the platelet response to thrombin. PAR1 is the high affinity thrombin receptor with rapid kinetics and a transient Ca^{2+} response which initiates the aggregation response, while **PAR4 is the low affinity thrombin receptor** with slower kinetics leading to a prolonged elevation in intracellular Ca^{2+} levels that sustains the irreversible platelet aggregation response [1]. PAR1 and PAR4 mediate different downstream signaling due to the different kinetics of their Ca^{2+} responses.

Furthermore, they can form heterodimers with other GPCRs involved in platelet activation, including adenosine P2Y12, α2-adrenergic, and β2 bradykinin receptors.

PAR4 antagonists are expected to have a better therapeutic window and lower bleeding risk than PAR1 antagonists when used in combination with other anti-platelet therapies, because unlike existing therapies that act on the initiation of coagulation, PAR4 acts at a later stage in the process [3]. Since the physiological contributions of PAR4 are most relevant under conditions of cell stress and inflammation, PAR4 antagonists are expected to primarily ameliorate pathological processes without significantly impacting necessary physiological processes.

Although a variety of PAR4 antagonists have been developed as tool compounds for research use, most lack the drug properties necessary for clinical development. BMS-986120 and BMS-986141 are optimized versions of PAR4 inhibitors identified in a small molecule screen. They were previously under clinical development for thrombosis by Bristol Myers Squibb.

Neuroprotective Benefit: PAR4 inhibition may mitigate thrombin-associated inflammation and neurovascular pathology.

Types of evidence:

- 1 genetic association study for PAR4 SNP and stroke
- Several laboratory studies for PAR4 biology

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function? None

Human research to suggest benefits to patients with dementia: None

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

Alzheimer's disease: Potential benefit based on thrombin biology

PAR4 antagonists have not yet been tested in the context of Alzheimer's disease (AD), but there is evidence to suggest that PAR4 inhibition could potentially protect against thromboinflammation associated pathology.

Thrombin plays a role in regulating CNS cells in a concentration dependent manner. Low concentrations are associated with neuroprotection, while high concentrations can promote

neurodegenerative processes [4]. Aβ42 has been shown to activate thrombin, suggesting that the buildup of amyloid promotes the neurotoxic effects of thrombin [5]. Brain derived microvessels and cerebrospinal fluid (CSF) samples from AD patients were found to express higher levels of thrombin than age-matched controls [6].

The PAR thrombin receptors are implicated in mediating the balance between neuroprotection and neurodegeneration by **regulating the inflammatory response**. The PARs can modulate the response to thrombin in the resident inflammatory mediators of the CNS, astrocytes and microglia [4]. In mice and rats, PAR4 was found to induce potent pro-inflammatory microgliosis, while PAR1 only had a minor stimulatory effect on the microglia [7]. **Caution is warranted in translating preclinical animal studies assessing PAR function, due to differences in PAR expression across species**, so verification of this finding in human tissue is needed. The discrepancy between PAR1 and PAR4 in microglial activation may stem from the finding that PAR4 levels are more dynamically regulated in response to inflammatory mediators [2]. Additionally, as a low-affinity thrombin receptor PAR4 is more likely to respond in the context of high thrombin levels, suggesting that PAR1 may mediate thrombin responses under basal conditions, whereas PAR4 activation may contribute to high thrombin associated pathology.

Aside from their roles in modulating CNS resident cells, PARs may contribute to neurovascular pathology in AD by modulating the activation status of platelets. Platelet activation increases in response to the presence of inflammatory mediators, which is thought to contribute to a feed-forward cycle of thromboinflammation [8]. The level of coated platelets, which are a subpopulation of platelets activated by collagen and thrombin that have high surface levels of procoagulant factors, was found to be correlated with disease progression in AD patients (r=-0.47) based on the change in Mini-Mental State Examination (MMSE) scores [9].

Stroke: Potential benefit based on genetic association of PAR4 and stroke

PAR4 expression is sensitive to the presence of inflammatory mediators and oxidative stress, which likely accounts for its upregulation in the context of cerebral ischemia. The alanine to threonine substitution stemming from a G to A single nucleotide polymorphism (SNP) at amino acid 120 of PAR4, is associated with increased PAR4 expression and platelet activation [10]. In a genetic association study (n=6255), there was a **modest effect size for increased risk for stroke** [Odds ratio (OR): 1.166, 95% confidence interval (CI) 1.006 to 1.356 for each A allele], and for large artery stroke (OR: 1.232, 95% CI 0.997 to1.524 for each A allele) **associated with the Thr120-A variant** [11].

The PAR4 antagonist, BMS-986141 (0.8 mg or 4.8 mg) in combination with aspirin (75 to 162 mg) for up to 28 days, was tested in a small placebo-controlled Phase 2 RCT (NCT02671461) (n=16) in patients who recently had a stroke or transient ischemic attack. The study was designed to assess the recurrence of stroke and bleeding risk following treatment; however, the outcome data was not analyzed due to study termination and the discontinuation of clinical development of this therapeutic agent.

In mice, PAR4 deficient mice had reduced cerebral infarct volume, neuronal death, BBB permeability, cerebral edema, and neurological impairments in the middle cerebral artery occlusion (MCAO) stroke model relative to wild-type mice [12]. The protective affects were attributed to decreased platelet activation and cerebral vascular inflammation. Due to species specific PAR expression, it is unclear how applicable these findings are to humans.

APOE4 interactions: Unknown

Aging and related health concerns: PAR4 inhibition may protect against thrombosis and diabetes-related cardiovascular complications.

Types of evidence:

- 2 clinical trials for BMS-986120 on *ex vivo* platelet responses in healthy volunteers
- 2 genetic-phenotype association studies for PAR4 SNP and platelet activation
- Several laboratory studies for PAR4 biology

Thrombosis: Potential benefit (*ex vivo* studies)

The PAR4 antagonists BMS-986120 and BMS-986141 were developed as anti-thrombotic agents. In the electrolytic carotid artery thrombosis (ECAT) model in cynomolgus monkeys, BMS-996120 and BMS-98141 reduced thrombosis weight by 82% (at 1 mg/kg) and 88% (at 0.5 mg/kg), respectively [13; 14]. These therapeutic agents reduced PAR4 induced platelet aggregation, but had minimal impact on hemostasis or bleeding risk. Similarly, ***ex vivo* PAR4 induced platelet aggregation was inhibited in healthy subjects treated with BMS-986120** (n=66) in a dose-dependent manner in a Phase 1 RCT [15]. In a separate Phase 1 trial in healthy male volunteers (NCT02439190) (n=40), BMS-986120 (60 mg) inhibited *ex vivo* PAR4 stimulated P-selectin expression by 92%, platelet aggregation by 85%, platelet-monocyte aggregates by 81%, and thrombus formation at a high shear rate, which is the fluid velocity divided by the distance from the vessel wall, by 29% [16]. The degree of inhibition was correlated with plasma levels of BMS-986120. Notably, BMS-986120 did not affect thrombus formation at low shear.

This distinction is significant because thrombus-prone arteries tend to have high shear stress, whereas bleeding prone vessels tend to have low shear rates [17].

SNPs in PAR4 are implicated in differential race-related cardiovascular event risk. A study comparing platelets from self-identified black (n=70) and white (n=84) participants found that, on average, platelets from black participants expressed 14% more PAR4 [10]. The Thr120-A variant SNP (rs773902) was found to be more common in black participants (63%), while the Ala120-G variant was more common in white participants (81%). **The Thr120-A variant was associated with higher PAR4 induced platelet aggregation and Ca^{2+} response.** Homozygotes for the Thr120-A variant showed 3-fold lower sensitivity to PAR4 desensitization relative to Ala120-G variant homozygotes [11]. Platelets from Thr120-A variant homozygotes also required over 2 times higher concentrations of the PAR4 antagonist YD-3 to achieve the same level of PAR4 inhibition, suggesting that the therapeutic window of PAR4 antagonists could be subject to a genotype effect [10]. However, the efficacy of BMS-986120 and related compound UDM-001651 was not influenced by this SNP in cell culture experiments [11; 18].

Diabetes: Potential benefit for cardiovascular inflammation (preclinical)

Diabetics have hyperreactive platelets, coagulation abnormalities, and impaired angiogenesis, suggesting a role for PAR4 overactivation [19]. **PAR4 expression is dynamically regulated, and increases in a variety of cell types in response to cell stressors, including high glucose conditions.** PAR4 was found to be upregulated in human smooth muscle cells and cardiac fibroblasts following exposure to high glucose [20; 21]. This **upregulation drives processes associated with adverse vascular and cardiac remodeling**, such as smooth muscle cell proliferation and migration, as well as the expression of pro-inflammatory mediators. While PAR1 levels were not affected, PAR4 was found to be increased in the right atrial appendages from patients with type 2 diabetes, and PAR4 expression was positively correlated with levels of IL-1β and cleaved caspase-1 [21].

PAR4 also promotes vascular inflammation by attracting immune cells. PAR4 mediated platelet activation promotes the release of leukocyte recruiting granules [22], while upregulation of PAR4 on monocytes, neutrophils, and leukocytes can trigger pro-inflammatory signaling [2]. The increase in circulating activated platelet-leukocyte aggregates in diabetic patients is consistent with an increase in PAR4 activation [19]. Although one study found that the *ex vivo* response to PAR4 was similar in platelets from diabetics and non-diabetics, the lack of a differential response may stem from the fact that glucose levels were well-controlled in this particular patient cohort [19].

Mouse models of diabetes have increased cardiovascular expression of PAR4, while PAR4 deficient mice are partially protected against diabetes-associated cardiovascular inflammation and remodeling [2]. In the high-fat diet induced model, cardiac upregulation of PAR4 drives the activation of the NLRP3 inflammasome, and downstream IL-1β mediated inflammation [21]. The loss of PAR4 prevented these adverse cardiac inflammatory processes in this model.

These studies suggest that while PAR4 may not significantly contribute to cardiovascular function in healthy individuals, it may contribute to cardiovascular inflammation in a clinically meaningful manner in the context of diabetes, such that PAR4 antagonists may offer therapeutic benefit.

Cancer: Potential benefit for cancer-associated thrombosis and colon cancer (preclinical)

The tumor microenvironment contains high levels of thrombin, and thrombin plays a role in supporting tumor growth and migration [23]. The activation of angiogenesis, which is essential for the growth of solid tumors, is one of the primary mechanisms by which thrombin favors tumor maintenance. Since the actions of thrombin in cancer are PAR mediated**, tumor cells can manipulate the expression of PARs to promote their survival**. By modulating the balance between PAR1 and PAR4, tumor cells can hijack the angiogenic properties of platelets. Platelets are known to secrete distinct subpopulations of granules. The granules can contain either pro-angiogenic or anti-angiogenic factors, and *ex vivo* studies have found that PAR1 stimulation drives secretion of pro-angiogenic granules, while PAR4 stimulation drives secretion of anti-angiogenic granules [24; 25]. Correspondingly, most cancers show a pattern where PAR1 expression is upregulated and PAR4 is downregulated. The expression of angiogenesis promoting PAR1 is correlated with malignant phenotype. Thrombin can also drive tumor growth through the release of growth factors and pro-survival signaling, which is at least in part, driven by the PAR driven activation of ERK1/2 [23]. **While in most tumors studied thus far, ERK1/2 mediated growth is driven by PAR1** [26]**, tumor growth and migration appear to be driven by PAR4 in colon cancer** [27]. Preclinical studies show that PAR4 promotes tumor cell apoptosis in a variety of cancer cell lines [28], aside from colon cancer. However, since PAR4 expression already tends to be low in most cancers, it is unclear whether treatment with a PAR4 antagonist would meaningfully contribute to tumor survival.

Aside from any potential influence on tumor growth, **PAR4 inhibitors may play a beneficial role in cancer-associated thrombosis**, which is the second leading cause of death in cancer patients [29]. Individuals with cancer are at 4 to 7 times higher risk for developing thrombosis, and cancer patients with a thrombolytic event have 2 to 3 times higher mortality risk [30]. As this bidirectional relationship suggests, platelets and tumor cells influence the behavior of one another. For example, platelet

activation has been shown to be increased in breast cancer patients, which is associated with higher risk for thrombocytosis [31]. Tumor cells can trigger platelet activation and aggregation. The platelet-tumor cell interactions can drive platelets to release procoagulant extracellular vesicles [32]. There is some evidence to suggest that PAR4 stimulated platelet-derived vesicles have the highest procoagulant potential [33]. Therefore, PAR4 antagonists could potentially mitigate the risk for cancer-associated thrombosis, though this hypothesis has not yet been clinically tested.

Pain: Potential tissue dependent effects for analgesia or exacerbation of pain

PAR4 is activated by serine proteases released in the context of injury and inflammation, such as cathepsin and trypsin, which contributes to pro-inflammatory signaling [1]. Studies assessing the role of PAR4 in nociception have produced conflicting results, as the injection of a PAR4 agonist into rat hind paw elicited an analgesic response [34], whereas it exacerbated the pain response when injected into the knee joint [35]. These studies suggest that the nociceptive properties of PAR4 may be tissue specific, as well as species specific.

Safety: May increase risk for bleeding, but to a lesser degree than existing anti-platelet therapies. Additional immunosuppressant effects or clinical population dependent effects are possible.

Types of evidence:

- 2 Phase 1 clinical trials for BMS-986120 in healthy volunteers
- 4 clinical trials for BMS-986141 (3 Phase 1 in healthy volunteers; 1 Phase 2 in stroke)
- Several laboratory studies

BMS-986120 (NCT02208882, NCT02439190) and BMS-986141 (NCT02341638, NCT02957448, NCT02922452) were tested in Phase 1 clinical trials in healthy volunteers. BMS-986120 was tested as a single oral dose (n=42) from 0.5 to 180 mg and in multiple doses (n=24) from 2.5 to 100 mg/day for 14 days [15]. The drug showed rapid absorption and distribution, with dose-related exposures. *Ex vivo* studies indicated robust inhibition of PAR4 induced platelet aggregation, but not PAR1 induced platelet activation. The incidence of treatment emergent adverse events was similar between BMS-986120 and placebo, and there were no drug-related discontinuations. BMS-986120 did not have any clinically meaningful impacts on routine coagulation tests or template bleeding times. In a separate study (NCT02439190) (n=40), BMS-986120 (60 mg oral dose) was administered 18 hours prior to the combination of aspirin and clopidogrel, and was found to be well-tolerated and produced no clinically significant effects on biochemical, hematologic, coagulation, physical, or electrocardiogram (ECG) safety measurements [16].

BMS-986141 was tested in single oral doses up to 150 mg and multiple doses up to 30 mg/day for 14 days, including a subset of participants who received BMS-986141 in combination with aspirin or the cytochrome P450 3A4 inhibitor itraconazole (n=148) (NCT02341638). There were **no clinically significant adverse events or findings on routine laboratory tests including coagulation profiles, template bleeding times, or liver enzyme tests** (Clinical Protocol CV006004). When combined with itraconazole, participants experienced up to 8-fold increased exposure of BMS-986141, and a 3-fold prolongation of half-life. In 28-day toxicity studies in monkeys, microscopic changes consistent with degeneration and regeneration in the kidney were observed with the highest dose (75 mg/kg/day).

PAR4 antagonists are expected to be associated with increased bleeding risks when used in combination with other anticoagulants. However, since PAR4 acts at a later step in the platelet activation and coagulation process relative to other available anti-platelet therapies, it is expected that the addition of a PAR4 antagonist will offer lower bleeding risk potential relative to currently used combinations. Most notably, PAR4 antagonists are expected to be safer than the PAR1 inhibitor, vorapaxar, which is limited by its very high bleeding risk when used in combination with standard of care therapy for thrombosis [3]. In cynomolgus monkeys, BMS-986141 (0.5 mg/kg), was associated with 1.2-fold increased bleeding time in the mesenteric artery, which is lower than the 2.2-fold increase for aspirin [14]. BMS-986141 in combination with aspirin increased bleeding time by 2.6 to 3-fold, which is far lower than the 8-fold increase in bleeding time for clopidogrel, which has been used as the standard of care.

Since PAR4 is induced in response to cellular stressors, its effects are context dependent, suggesting that the side effect profile could vary in different clinical populations. PAR4 deficient mice do not show spontaneous phenotypes, aside from minor bleeding, since PAR4 has minimal contribution to the activity of non-platelet cells under basal conditions, but phenotypes emerge in response to physiological challenges, when PAR4 activity becomes biologically relevant [3]. Due to its role in inflammation and injury responses, PAR4 inhibition could potentially increase the risk for infections by dampening the immune system [2].

Sources and dosing:

BMS-986120 is available for research use from commercial suppliers, but clinical development has been discontinued. It had been administered orally in clinical trials, and doses ≥10 mg could fully inhibit *ex vivo* PAR4 mediated platelet aggregation and activation.

Research underway:

Bristol Myers Squibb has discontinued clinical development of BMS-986120 and BMS-986141 for unknown reasons. There are several academic labs working to develop new PAR4 antagonists, including derivatives of BMS-986120, with improved metabolic stability and pharmacokinetic properties [36; 37; 38].

Search terms:

Pubmed, Google: BMS-986120, BMS-986141, PAR4 Antagonist

- Alzheimer's, neurodegeneration, stroke, inflammation, thrombosis, cardiovascular, diabetes, cancer, clinical trial, safety

Websites visited for BMS-986120 & BMS-986141:

- Clinicaltrials.gov BMS-986120, BMS-986141
- PubChem BMS-986120, BMS-986141
- DrugBank.ca BMS-986141

References:

1. French SL, Hamilton JR (2016) Protease-activated receptor 4: from structure to function and back again. *Br J Pharmacol* 173, 2952-2965.https://bpspubs.onlinelibrary.wiley.com/doi/abs/10.1111/bph.13455

2. Fender AC, Rauch BH, Geisler T *et al.* (2017) Protease-Activated Receptor PAR-4: An Inducible Switch between Thrombosis and Vascular Inflammation? *Thromb Haemost* 117, 2013-2025.https://www.ncbi.nlm.nih.gov/pubmed/29044290

3. Li S, Tarlac V, Hamilton JR (2019) Using PAR4 Inhibition as an Anti-Thrombotic Approach: Why, How, and When? *International Journal of Molecular Sciences* 20, 5629.https://www.mdpi.com/1422-0067/20/22/5629

4. Rohatgi T, Sedehizade F, Reymann KG *et al.* (2004) Protease-Activated Receptors in Neuronal Development, Neurodegeneration, and Neuroprotection: Thrombin as Signaling Molecule in the Brain. *The Neuroscientist* 10, 501-512.https://journals.sagepub.com/doi/abs/10.1177/1073858404269955

5. De Luca C, Colangelo AM, Alberghina L *et al.* (2018) Neuro-Immune Hemostasis: Homeostasis and Diseases in the Central Nervous System. *Front Cell Neurosci* 12, 459-459.https://www.ncbi.nlm.nih.gov/pubmed/30534057 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6275309/

6. Yin X, Wright J, Wall T *et al.* (2010) Brain endothelial cells synthesize neurotoxic thrombin in Alzheimer's disease. *Am J Pathol* 176, 1600-1606.https://www.ncbi.nlm.nih.gov/pubmed/20150433 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2843451/

7. Suo Z, Wu M, Citron BA *et al.* (2003) Persistent Protease-activated Receptor 4 Signaling Mediates Thrombin-induced Microglial Activation. *Journal of Biological Chemistry* 278, 31177-31183.http://www.jbc.org/content/278/33/31177.abstract

8. Espinosa-Parrilla Y, Gonzalez-Billault C, Fuentes E *et al.* (2019) Decoding the Role of Platelets and Related MicroRNAs in Aging and Neurodegenerative Disorders. *Front Aging Neurosci* 11, 151-151.https://www.ncbi.nlm.nih.gov/pubmed/31312134 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6614495/

9. Prodan CI, Ross ED, Vincent AS *et al.* (2008) Rate of progression in Alzheimer's disease correlates with coated-platelet levels—a longitudinal study. *Translational Research* 152, 99-102.http://www.sciencedirect.com/science/article/pii/S1931524408001874

10. Edelstein LC, Simon LM, Lindsay CR *et al.* (2014) Common variants in the human platelet PAR4 thrombin receptor alter platelet function and differ by race. *Blood* 124, 3450-3458.https://www.ncbi.nlm.nih.gov/pubmed/25293779 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4246040/

11. Whitley MJ, Henke DM, Ghazi A *et al.* (2018) The protease-activated receptor 4 Ala120Thr variant alters platelet responsiveness to low-dose thrombin and protease-activated receptor 4 desensitization, and is blocked by non-competitive P2Y(12) inhibition. *J Thromb Haemost* 16, 2501-2514.https://www.ncbi.nlm.nih.gov/pubmed/30347494 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6289679/

12. Mao Y, Zhang M, Tuma RF *et al.* (2010) Deficiency of PAR4 attenuates cerebral ischemia/reperfusion injury in mice. *J Cereb Blood Flow Metab* 30, 1044-1052.https://www.ncbi.nlm.nih.gov/pubmed/20087365 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2949190/

13. Wong PC, Seiffert D, Bird JE *et al.* (2017) Blockade of protease-activated receptor-4 (PAR4) provides robust antithrombotic activity with low bleeding. *Science Translational Medicine* 9, eaaf5294.https://stm.sciencemag.org/content/scitransmed/9/371/eaaf5294.full.pdf

14. Wong PC, Watson CA, Bostwick J *et al.* (2017) Abstract 13794: An Orally-Active Small-Molecule Antagonist of the Platelet Protease-Activated Receptor-4, BMS-986141, Prevents Aeterial Thrombosis With Low Bleeding Liability in Cynomolgus Monkeys. *Circulation* 136, A13794-A13794.https://ahajournals.org/doi/abs/10.1161/circ.136.suppl_1.13794

15. Ismat FA, Ma X, Wang Z *et al.* (2016) Abstract TMP91: Phase I Assessment of the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of the Oral Protease-activated Receptor-4 Antagonist BMS-986120. *Stroke* 47, ATMP91-ATMP91.https://www.ahajournals.org/doi/abs/10.1161/str.47.suppl_1.tmp91

16. Wilson SJ, Ismat FA, Wang Z *et al.* (2018) PAR4 (Protease-Activated Receptor 4) Antagonism With BMS-986120 Inhibits Human Ex Vivo Thrombus Formation. *Arterioscler Thromb Vasc Biol* 38, 448-456.https://www.ncbi.nlm.nih.gov/pubmed/29269513 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5779320/

17. Casa LDC, Deaton DH, Ku DN (2015) Role of high shear rate in thrombosis. *Journal of Vascular Surgery* 61, 1068-1080.http://www.sciencedirect.com/science/article/pii/S0741521415000221

18. Callejo MF, Colin J, Desmeules S *et al.* (2016) Abstract WP263: Mutagenesis Studies Revealed Minimal Impact of Human A120T Variant of Protease-activated Receptor 4 on Receptor function or Pharmacological Response to a Potent and Selective Antagonist. *Stroke* 47, AWP263-AWP263.https://www.ahajournals.org/doi/abs/10.1161/str.47.suppl_1.wp263

19. Miao X, Zhang W, Huang Z *et al.* (2016) Unaltered Angiogenesis-Regulating Activities of Platelets in Mild Type 2 Diabetes Mellitus despite a Marked Platelet Hyperreactivity. *PLOS ONE* 11, e0162405.https://doi.org/10.1371/journal.pone.0162405

20. Dangwal S, Rauch BH, Gensch T *et al.* (2011) High Glucose Enhances Thrombin Responses via Protease-Activated Receptor-4 in Human Vascular Smooth Muscle Cells. *Arterioscler Thromb Vasc Biol* 31, 624-633.https://www.ahajournals.org/doi/abs/10.1161/ATVBAHA.110.219105

21. Fender AC, Kleeschulte S, Stolte S *et al.* (2020) Thrombin receptor PAR4 drives canonical NLRP3 inflammasome signaling in the heart. *Basic Research in Cardiology* 115, 10.https://doi.org/10.1007/s00395-019-0771-9

22. Rigg RA, Healy LD, Chu TT *et al.* (2019) Protease-activated receptor 4 activity promotes platelet granule release and platelet-leukocyte interactions. *Platelets* 30, 126-135.https://www.ncbi.nlm.nih.gov/pubmed/30560697 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6397092/

23. Han N, Jin K, He K *et al.* (2011) Protease-activated receptors in cancer: A systematic review. *Oncol Lett* 2, 599-608.https://www.ncbi.nlm.nih.gov/pubmed/22848234 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3406414/

24. Ma L, Perini R, McKnight W *et al.* (2005) Proteinase-activated receptors 1 and 4 counter-regulate endostatin and VEGF release from human platelets. *Proceedings of the National Academy of Sciences of the United States of America* 102, 216-220.https://www.pnas.org/content/pnas/102/1/216.full.pdf

25. Italiano JE, Jr., Richardson JL, Patel-Hett S *et al.* (2008) Angiogenesis is regulated by a novel mechanism: pro- and antiangiogenic proteins are organized into separate platelet alpha granules and differentially released. *Blood* 111, 1227-1233.https://www.ncbi.nlm.nih.gov/pubmed/17962514 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2214735/

26. Liu X, Yu J, Song S *et al.* (2017) Protease-activated receptor-1 (PAR-1): a promising molecular target for cancer. *Oncotarget* 8, 107334-107345.https://www.ncbi.nlm.nih.gov/pubmed/29291033 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5739818/

27. Zhang H, Jiang P, Zhang C *et al.* (2018) PAR4 overexpression promotes colorectal cancer cell proliferation and migration. *Oncol Lett* 16, 5745-5752.https://www.ncbi.nlm.nih.gov/pubmed/30333860 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6176407/

28. Jiang P, De Li S, Li ZG *et al.* (2018) The expression of protease-activated receptors in esophageal carcinoma cells: the relationship between changes in gene expression and cell proliferation, apoptosis in vitro and growing ability in vivo. *Cancer Cell International* 18, 81.https://doi.org/10.1186/s12935-018-0577-0

29. Sheth RA, Niekamp A, Quencer KB *et al.* (2017) Thrombosis in cancer patients: etiology, incidence, and management. *Cardiovasc Diagn Ther* 7, S178-S185.https://www.ncbi.nlm.nih.gov/pubmed/29399521 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5778519/

30. Sharma BK, Flick MJ, Palumbo JS (2019) Cancer-Associated Thrombosis: A Two-Way Street. *Semin Thromb Hemost* 45, 559-568.https://www.ncbi.nlm.nih.gov/pubmed/31382306

31. Holmes CE, Levis JE, Schneider DJ *et al.* (2016) Platelet phenotype changes associated with breast cancer and its treatment. *Platelets* 27, 703-711.https://doi.org/10.3109/09537104.2016.1171302

32. Mitrugno A, Yunga ST, Sylman JL *et al.* (2019) The role of coagulation and platelets in colon cancer-associated thrombosis. *American Journal of Physiology-Cell Physiology* 316, C264-C273.https://www.physiology.org/doi/abs/10.1152/ajpcell.00367.2018

33. Duvernay M, Young S, Gailani D *et al.* (2013) Protease-activated receptor (PAR) 1 and PAR4 differentially regulate factor V expression from human platelets. *Mol Pharmacol* 83, 781-792.https://www.ncbi.nlm.nih.gov/pubmed/23307185 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3608438/

34. Asfaha S, Cenac N, Houle S *et al.* (2007) Protease-activated receptor-4: a novel mechanism of inflammatory pain modulation. *Br J Pharmacol* 150, 176-185.https://www.ncbi.nlm.nih.gov/pubmed/17179954 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2042908/

35. Russell FA, Veldhoen VE, Tchitchkan D *et al.* (2010) Proteinase-Activated Receptor-4 (PAR4) Activation Leads to Sensitization of Rat Joint Primary Afferents Via a Bradykinin B2 Receptor-Dependent Mechanism. *Journal of Neurophysiology* 103, 155-163.https://www.physiology.org/doi/abs/10.1152/jn.00486.2009

36. Chen P, Ren S, Song H *et al.* (2019) Synthesis and biological evaluation of BMS-986120 and its deuterated derivatives as PAR4 antagonists. *Bioorganic & Medicinal Chemistry* 27, 116-124.http://www.sciencedirect.com/science/article/pii/S0968089618316389

37. Moschonas IC, Kellici TF, Mavromoustakos T *et al.* (2017) Molecular requirements involving the human platelet protease-activated receptor-4 mechanism of activation by peptide analogues of its tethered-ligand. *Platelets* 28, 812-821.https://doi.org/10.1080/09537104.2017.1282607

38. Temple KJ, Duvernay MT, Young SE *et al.* (2016) Development of a Series of (1-Benzyl-3-(6-methoxypyrimidin-3-yl)-5-(trifluoromethoxy)-1H-indol-2-yl)methanols as Selective Protease Activated Receptor 4 (PAR4) Antagonists with in Vivo Utility and Activity Against γ-Thrombin. *Journal of medicinal chemistry* 59, 7690-7695.https://www.ncbi.nlm.nih.gov/pubmed/27482618 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5775816/