

At the MERcy of platelet primers

Citation for published version (APA):

Cosemans, J. M. E. M. (2018). At the MERcy of platelet primers. *Journal of Thrombosis and Haemostasis*, 16(2), 349-351. <https://doi.org/10.1111/jth.13915>

Document status and date:

Published: 01/02/2018

DOI:

[10.1111/jth.13915](https://doi.org/10.1111/jth.13915)

Document Version:

Publisher's PDF, also known as Version of record

Document license:

Taverne

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

COMMENTARY

At the MERcy of platelet primers

J. M. E. M. COSEMANS

Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, the Netherlands

To cite this article: Cosemans JMEM. At the MERcy of platelet primers. *J Thromb Haemost* 2018; **16**: 349–51.

See also Branchford BR, Stalker TJ, Law L, Acevedo G, Sather S, Brzezinski C, Wilson KM, Minson K, Lee-Sherick AB, Davizon-Castillo P, Ng C, Zhang W, Neeves KB, Lentz SR, Wang X, Frye SV, Shelton Earp H III, DeRyckere D, Brass LF, Graham DK, Di Paola JA. The small-molecule MERTK inhibitor UNC2025 decreases platelet activation and prevents thrombosis. This issue, pp 352–63.

In this issue of the *Journal of Thrombosis and Haemostasis*, Branchford *et al.* [1] present novel data on the role of Mer tyrosine kinase in platelet function and thrombosis. Mer forms, together with Axl and Tyro3, the TAM receptor family of growth arrest-specific (Gas) receptors. The vitamin K-dependent protein Gas6 is regarded as a platelet primer, like other molecules such as epinephrine, insulin-like growth factor-1, insulin-like growth factor-2, matrix metalloproteinase-1, matrix metalloproteinase-2, oxidized LDL, prostaglandin E2, soluble CD40 ligand, and thrombopoietin [2]. Platelet primers are platelet agonists that are unable to stimulate platelets by themselves, but potentiate activation processes evoked by other agonists. Gas6 is released by a number of cell types, including endothelial cells, vascular smooth muscle cells, bone marrow, and, to a limited extent, human platelets (reviewed in [3]). Binding of Gas6 to its receptors is thought to result in dimerization of two receptor monomers, which provokes autophosphorylation of tyrosines in the cytosolic moieties of the receptor. Intracellular signaling via phosphatidylinositol 3-kinase (PI3K) isoforms plays a central role in mediating the effects of Gas6 and other platelet primers, with different PI3K isoforms having divergent roles. Gas6 levels are elevated in systemic inflammatory disorders, such as sepsis and lupus erythematosus, and in venous thromboembolic disease (reviewed in [3]). Healthy subjects with elevated Gas6 levels have been found to have an approximately 10-fold higher

risk of being aspirin-pseudoresistant, i.e. subject to persistent platelet aggregation despite inhibition of thromboxane A2 production by aspirin [4]. The latter suggests that priming of platelets might be a mechanism of resistance to antiplatelet therapy.

It is a topic of debate whether only Mer is present on the surfaces of human and mouse platelets [5,6], or whether Axl and Tyro3 are also present [7–10]. It is conceivable that Axl and Tyro3 are expressed at such low levels that they remain below the detection limit of some of the assays used. Unfortunately, even highly sensitive platelet proteomics studies have failed to shed light on this matter, because, in contrast to what has been found in other cell types [11], neither the TAM receptors nor Gas6 could be detected in human or mouse platelets [12,13]. Interestingly, however, knocking out *Mer*, *Axl* or *Tyro3* in mice yields a moderate antithrombotic effect [8]. TAM receptors also modulate inflammation and cell growth, and, importantly, appear to have distinct roles in these processes. *Mer*^{-/-} mice show several autoimmune-like features, whereas *Tyro3*^{-/-} mice develop neurologic defects, and *Axl*^{-/-} mice have vascular defects similar to those seen in *Gas6*^{-/-} mice (reviewed in [3]). In addition, Axl is predominantly overexpressed in a variety of human cancers [14].

How important is Mer in mediating thrombosis and hemostasis? Branchford *et al.* [1] thoroughly addressed this question by investigating the effects of a small-molecule inhibitor of Mer, UNC2025, on platelet function and thrombosis. It should be noted that UNC2025 is equally potent in inhibiting fms-related tyrosine kinase 3 (FLT3) as in inhibiting Mer. However, FLT3 has not been reported in human or mouse megakaryocytes or platelets. The results of Branchford *et al.* [1] clearly demonstrate that UNC2025 has antithrombotic characteristics both *in vitro* and *in vivo* in an arterial and venous thrombosis model, without increasing tail bleeding. These findings are in line with previous observations made in *Mer*^{-/-} mice [5,8]. Interestingly, using an adapted tail bleeding protocol, Angelillo-Scherrer *et al.* [8] demonstrated that *Mer*^{-/-} mice show a tendency to rebleed after initial cessation of

Correspondence: Judith M. E. M. Cosemans, Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, the Netherlands
Tel.: + 31 43 388 1534
E-mail: Judith.cosemans@maastrichtuniversity.nl

Received: 22 November 2017

Manuscript handled by: P. H. Reitsma

Final decision: P. H. Reitsma, 22 November 2017

bleeding, whereas initial bleeding times are unaltered. How this information can be applied to hemostasis in humans is still unclear. Importantly, UNC2025 inhibits phosphorylation of Mer, indicating a specific effect of the compound on its target receptor, and of Akt and Src. Although Akt and Src are recognized downstream targets of Mer [3], a direct effect of UNC2025 on PI3K or Src, or via a platelet receptor other than Mer, cannot be ruled out completely. A key functional effect of signaling via Mer, and the other TAM receptors, is continued activation of $\alpha_{IIb}\beta_3$ [8,10]. Integrin $\alpha_{IIb}\beta_3$ activation is reversible, and impaired activation of this receptor causes instability of thrombi that are formed under flow *in vivo* (mouse) or *in vitro* (human and mouse), resulting in detachment of single platelets and small aggregates (reviewed in [15]). Similarly to observations made with (blood from) *Mer*^{-/-} mice [8,10], treatment with UNC2025 resulted in reduced aggregate stability of human thrombi under flow. The combination of UNC2025 with low doses of a P2Y₁₂ inhibitor yielded a synergistic antiplatelet effect, both *in vitro* with human platelets, and *in vivo* in an arterial and venous thrombosis model. Intriguingly, this synergistic effect is not accompanied by an increase in tail bleeding time, such as is seen with high doses of P2Y₁₂ inhibitor [1]. Whether this combined treatment affects rebleeding has not been investigated yet.

How can a synergistic antithrombotic effect of UNC2025 on top of a low dose of P2Y₁₂ inhibitor without a concomitant increase in bleeding be explained? First, although Gas6 and P2Y₁₂ synergize at the level of Akt phosphorylation [10], platelet signaling pathways downstream of Mer and P2Y₁₂ may differ with respect to the temporal regulation of PI3K and Akt signaling and/or involve different downstream mediators. Support for different temporal regulation comes from the observation that Gas6–Gas6 receptor interaction becomes progressively more important at later stages, i.e. when the ADP pathway is reduced in activity or desensitizes [10]. Second, it appears that both P2Y₁₂ and Mer are also expressed in endothelial cells and leukocytes, at least at the mRNA level [3,16], indicating that multiple cell types may be involved in the thrombotic action of P2Y₁₂ and Mer. To exemplify this further, Gas6 has been demonstrated to upregulate tissue factor in endothelial cells upon vessel wall injury, leading to activation of the extrinsic coagulation pathway and to thrombus formation in mice [17]. Gas6 also triggers expression of the adhesion molecules ICAM-1 and VCAM-1 on endothelial cells, promoting platelet–endothelium and leukocyte–endothelium interactions [18], which suggests a role for Gas6 in platelet adhesion and activation under inflammatory conditions. Although *in vitro* flow chamber studies and experimental thrombosis models have provided valuable insights into the underlying mechanism of initial platelet interaction with activated endothelium, it remains obscure how thrombus formation and stabilization is regulated under these conditions [19].

How can the hemostatic effects of platelet Mer be dissected from those of endothelial Mer? A logical approach would be to use transgenic mouse models harbouring endothelial-specific or platelet-specific deletions of Mer. Alternatively, *Mer*^{-/-} platelets could be transfused into mice that allow adoptive platelet transfer, e.g. human interleukin-4 receptor- α /glycoprotein Ib α -transgenic mice. The potential synergistic action of Mer and P2Y₁₂ could be investigated in these models by injecting *Mer*^{-/-} or *P2yr12*^{-/-} mice with, respectively, a P2Y₁₂ inhibitor or UNC2025 or, vice versa, by infusing inhibitor-treated platelets into donor mice. In addition, it will be of interest to investigate the effect of a platelet-specific or endothelial-specific deletion of Mer in mouse models of systemic inflammation, regardless of whether plasma Gas6 is elevated in these models or not. A potential complicating factor is that, besides Gas6, the following other ligands have been reported to bind to and activate Mer: protein S, Tubby, Tulp1, and galectin-3. Protein S serves as an anticoagulant predominantly by working as a non-enzymatic cofactor for activated protein C in the breakdown of activated factor V and activated factor VIII. Although human protein S has only weak or no affinity for the different human TAM receptors, there are reports indicating that human protein S affects Mer function in human macrophages (reviewed in [3,14]). So far, Tubby, Tulp1 and galectin-3 have been shown to be involved in Mer receptor-mediated efferocytosis (reviewed in [3]). Whether these ligands can affect hemostasis via Mer remains to be elucidated.

With regard to UNC2025, it is essential to further examine potential side effects, in particular those resulting from long-term treatment. So far, it has been shown that treatment with 75 mg/kg UNC2025 for several weeks leads to anemia and leukopenia in mice [20]. It is of note that a 25-fold lower dose of UNC2025 sufficed to fulfil an antithrombotic function in mice [1]. Given that UNC2025 is a Mer/FLT3 dual inhibitor, and that specific inhibition of FLT3 [21], but not of a loss of function of Mer [22], leads to similar defects in hematopoiesis as a high dose of UNC2025, it is likely that the reported side effects are mediated via inhibition of FLT3.

Together, the above findings indicate that the divergent roles of TAM receptors in hemostasis, inflammation and cell growth warrant careful study of potential adverse side effects when a TAM receptor is targeted as a therapeutic approach. However, they may also create opportunities to target pathologies that lie at the interface of these biological processes, e.g. inflammation-induced thrombosis. Patients who respond poorly to conventional antiplatelet therapy and have elevated plasma levels of platelet primers, such as Gas6, may benefit from treatment with a TAM inhibitor on top of standard medication. Moreover, if the synergistic effect of Mer and P2Y₁₂ on thrombosis, but not bleeding, holds true for humans, combined treatment in patients in need of antithrombotic medication but with a relatively high

bleeding risk may prove more beneficial than standard antiplatelet treatment.

Acknowledgements

The author receives research funding from the Dutch Heart Foundation (2015T79) and the Netherlands Organisation for Scientific Research (NWO Vidi 91716421).

Disclosure of Conflict of Interests

The author states that she has no conflict of interest.

References

- Branchford BR, Stalker TJ, Law L, Acevedo G, Sather S, Brzezinski C, Wilson KM, Minson K, Lee-Sherick AB, Davizon-Castillo P, Ng C, Zhang W, Neeves KB, Lentz SR, Wang X, Frye SV, Shelton Earp H III, DeRyckere D, Brass LF, Graham DK, Di Paola JA. The small molecule MERTK inhibitor UNC2025 decreases platelet activation and prevents thrombosis. *J Thromb Haemost* 2017; <https://doi.org/10.1111/jth.13875>.
- Baaten C, Ten Cate H, van der Meijden PEJ, Heemskerk JWM. Platelet populations and priming in hematological diseases. *Blood Rev* 2017; **31**: 389–99.
- van der Meer JH, van der Poll T, van't Veer C. TAM receptors, Gas6, and protein S: roles in inflammation and hemostasis. *Blood* 2014; **123**: 2460–9.
- Burnier L, Borgel D, Angelillo-Scherrer A, Fontana P. Plasma levels of the growth arrest-specific gene 6 product (Gas6) and antiplatelet drug responsiveness in healthy subjects. *J Thromb Haemost* 2006; **4**: 2283–4.
- Chen C, Li Q, Darrow AL, Wang Y, Derian CK, Yang J, de Garavilla L, Andrade-Gordon P, Damiano BP. Mer receptor tyrosine kinase signaling participates in platelet function. *Arterioscler Thromb Vasc Biol* 2004; **24**: 1118–23.
- Uras F, Kucuk B, Bingol Ozakpinar O, Demir AM. Growth arrest-specific 6 (Gas6) and TAM receptors in mouse platelets. *Turk J Haematol* 2015; **32**: 58–63.
- Angelillo-Scherrer A, de Frutos P, Aparicio C, Melis E, Savi P, Lupu F, Arnout J, Dewerchin M, Hoylaerts M, Herbert J, Collen D, Dahlbäck B, Carmeliet P. Deficiency or inhibition of Gas6 causes platelet dysfunction and protects mice against thrombosis. *Nat Med* 2001; **7**: 215–21.
- Angelillo-Scherrer A, Burnier L, Flores N, Savi P, DeMol M, Schaeffer P, Herbert JM, Lemke G, Goff SP, Matsushima GK, Earp HS, Vesin C, Hoylaerts MF, Plaisance S, Collen D, Conway EM, Wehrle-Haller B, Carmeliet P. Role of Gas6 receptors in platelet signaling during thrombus stabilization and implications for antithrombotic therapy. *J Clin Invest* 2005; **115**: 237–46.
- Gould WR, Baxi SM, Schroeder R, Peng YW, Leadley RJ, Peterson JT, Perrin LA. Gas6 receptors Axl, Sky and Mer enhance platelet activation and regulate thrombotic responses. *J Thromb Haemost* 2005; **3**: 733–41.
- Cosemans JM, Van Kruchten R, Olieslagers S, Schurgers LJ, Verheyen FK, Munnix IC, Waltenberger J, Angelillo-Scherrer A, Hoylaerts MF, Carmeliet P, Heemskerk JW. Potentiating role of Gas6 and Tyro3, Axl and Mer (TAM) receptors in human and murine platelet activation and thrombus stabilization. *J Thromb Haemost* 2010; **8**: 1797–808.
- Twoorkoski K, Singhal G, Szpakowski S, Zito CI, Bacchicocchi A, Muthusamy V, Bosenberg M, Krauthammer M, Halaban R, Stern DF. Phosphoproteomic screen identifies potential therapeutic targets in melanoma. *Mol Cancer Res* 2011; **9**: 801–12.
- Burkhart JM, Vaudel M, Gambaryan S, Radau S, Walter U, Martens L, Geiger J, Sickmann A, Zahedi RP. The first comprehensive and quantitative analysis of human platelet protein composition allows the comparative analysis of structural and functional pathways. *Blood* 2012; **120**: e73–82.
- Zeiler M, Moser M, Mann M. Copy number analysis of the murine platelet proteome spanning the complete abundance range. *Mol Cell Proteomics* 2014; **13**: 3435–45.
- Hafizi S, Dahlback B. Gas6 and protein S. Vitamin K-dependent ligands for the Axl receptor tyrosine kinase subfamily. *FEBS J* 2006; **273**: 5231–44.
- Cosemans JM, Iserbyt BF, Deckmyn H, Heemskerk JW. Multiple ways to switch platelet integrins on and off. *J Thromb Haemost* 2008; **6**: 1253–61.
- Liverani E, Rico MC, Tsygankov AY, Kilpatrick LE, Kunapuli SP. P2Y12 receptor modulates sepsis-induced inflammation. *Arterioscler Thromb Vasc Biol* 2016; **36**: 961–71.
- Robins RS, Lemarie CA, Laurance S, Aghourian MN, Wu J, Blostein MD. Vascular Gas6 contributes to thrombogenesis and promotes tissue factor up-regulation after vessel injury in mice. *Blood* 2013; **121**: 692–9.
- Tjwa M, Bellido-Martin L, Lin Y, Lutgens E, Plaisance S, Bono F, Delesque-Touchard N, Herve C, Moura R, Billiau AD, Aparicio C, Levi M, Daemen M, Dewerchin M, Lupu F, Arnout J, Herbert JM, Waer M, Garcia de Frutos P, Dahlback B, et al. Gas6 promotes inflammation by enhancing interactions between endothelial cells, platelets, and leukocytes. *Blood* 2008; **111**: 4096–105.
- Coenen DM, Mastenbroek TG, Cosemans J. Platelet interaction with activated endothelium: mechanistic insights from microfluidics. *Blood* 2017; in press. <https://doi.org/10.1182/blood-2017-04-780825>.
- DeRyckere D, Lee-Sherick AB, Huey MG, Hill AA, Tyner JW, Jacobsen KM, Page LS, Kirkpatrick GG, Eryildiz F, Montgomery SA, Zhang W, Wang X, Frye SV, Earp HS, Graham DK. UNC2025, a MERTK small-molecule inhibitor, is therapeutically effective alone and in combination with methotrexate in leukemia models. *Clin Cancer Res* 2017; **23**: 1481–92.
- Perl AE, Altman JK, Cortes J, Smith C, Litzow M, Baer MR, Claxton D, Erba HP, Gill S, Goldberg S, Jurcic JG, Larson RA, Liu C, Ritchie E, Schiller G, Spira AI, Strickland SA, Tibes R, Ustun C, Wang ES, et al. Selective inhibition of FLT3 by gilteritinib in relapsed or refractory acute myeloid leukaemia: a multicentre, first-in-human, open-label, phase 1–2 study. *Lancet Oncol* 2017; **18**: 1061–75.
- Parinot C, Nandrot EF. A comprehensive review of mutations in the MERTK proto-oncogene. *Adv Exp Med Biol* 2016; **854**: 259–65.