



HAL
open science

Glenzocimab does not impact glycoprotein VI-dependent inflammatory haemostasis

Soumaya Jadoui, Ophélie Le Chapelain, Véronique Ollivier, Ali Mostefa-Kara,
Lucas Di Meglio, Sébastien Dupont, Angèle Gros, Mialitiana Solo
Nomenjanahary, Jean-Philippe Desilles, Mikaël Mazighi, et al.

► **To cite this version:**

Soumaya Jadoui, Ophélie Le Chapelain, Véronique Ollivier, Ali Mostefa-Kara, Lucas Di Meglio, et al..
Glenzocimab does not impact glycoprotein VI-dependent inflammatory haemostasis. *Haematologica*,
Ferrata Storti Foundation, 2020, Online ahead of print. 10.3324/haematol.2020.270439 . inserm-
03100131

HAL Id: inserm-03100131

<https://www.hal.inserm.fr/inserm-03100131>

Submitted on 6 Jan 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Glenzocimab does not impact glycoprotein VI-dependent inflammatory haemostasis

Running head: Glenzocimab and inflammatory haemostasis

Soumaya Jadoui^{1*}, Ophélie Le Chapelain^{1*}, Véronique Ollivier¹, Ali Mostefa-Kara¹, Lucas Di Meglio¹, Sébastien Dupont¹, Angèle Gros¹, Mialitiana Solo Nomenjanahary¹, Jean-Philippe Desilles^{1,2}, Mikaël Mazighi^{1,2}, Bernhard Nieswandt³, Stéphane Loyau¹, Martine Jandrot-Perrus¹, Pierre H Mangin⁴, Benoit Ho-Tin-Noé^{1#}.

¹*Université de Paris, LVTS, Inserm U1148, F-75018 Paris, France.*

²*Rothschild Foundation Hospital, Paris, France. Department of Interventional Neuroradiology.*

³*University Hospital Würzburg, Rudolf Virchow Center for Experimental Biomedicine, Würzburg, Germany.*

⁴*Université de Strasbourg, INSERM, EFS Grand-Est, BPPS UMR-S1255, FMTS, F-67065 Strasbourg, France.*

*equal contribution

#Correspondence:

Benoît Ho-Tin-Noé, PhD.

Laboratory of Vascular Translational Science, INSERM U1148, Hôpital Bichat, 46 rue Henri Huchard

75877 Paris Cedex 18 France.

Fax: + 33 (0) 1 40 25 86 02.

Tel: + 33 (0) 1 40 25 86 00.

benoit.ho-tin-noe@inserm.fr

Glycoprotein VI (GPVI), the main platelet receptor for collagen, has emerged as a new target for antithrombotic therapy because its genetic deficiency or pharmacological blocking inhibits platelet aggregation and experimental thrombosis without increasing bleeding time¹⁻⁵. While these data have stimulated the development of new antiplatelet drugs targeting GPVI, recent findings have indicated that GPVI is essential for repair of neutrophil-induced vascular injury in various inflamed organs and tissues⁶⁻⁹. It thus appears important to assess and anticipate the yet uninvestigated risk of inflammation-induced bleeding under GPVI antagonists, especially considering that inflammation is a component of various thrombotic diseases. In that respect, it is worth noting that neutrophil mobilization is a predictor of hemorrhagic transformation of ischemic stroke¹⁰ and contributes to intraplaque hemorrhage¹¹, which is known to precipitate plaque rupture and the clinical expression of atherosclerosis.

Among the newly developed drugs targeting GPVI, ACT017 (Glenzocimab, Acticor Biotech) is a humanized antibody fragment (Fab) that has already completed its phase I clinical trial in healthy volunteers¹² and just entered a phase II trial in stroke patients (Acute Ischemic Stroke Interventional Study “ACTIMIS”, NCT03803007). ACT017 binds to human GPVI and inhibits the procoagulant activity and aggregation of collagen-stimulated platelets, as well as platelet adhesion and thrombus formation onto collagen surfaces under arterial flow conditions^{1,13,14}. The inhibitory action of ACT017 occurs without causing thrombocytopenia or depletion of GPVI, and is not associated with spontaneous bleeding events or increased bleeding time¹⁴. Nevertheless, whereas preclinical bleeding time tests can help evaluate the risk of bleeding associated with trauma or surgery, they may not predict the risk of bleeding associated with inflammation¹⁵. Here, using the cutaneous reverse passive Arthus reaction (rpA) as a model situation where GPVI plays a major role in inflammatory haemostasis, we investigated whether ACT017 increases the risk of inflammation-induced bleeding.

We first assessed the contribution of GPVI to the prevention of inflammation-induced bleeding by platelets in the brain and lungs. In agreement with previous results obtained with an antibody causing depletion of mouse GPVI^{16,17}, there was no cerebral haemorrhage in any of the GPVI^{-/-} mice subjected to transient (90 min) middle cerebral artery occlusion (Figure 1A). In contrast, cerebral haemorrhage occurred in all mice that had been rendered severely thrombocytopenic by the mean of a platelet-depleting antibody (Figure 1A). Genetic deficiency in GPVI was not associated with an increased bleeding risk in the model of acute lung injury induced by inhalation of *P. aeruginosa* endotoxin either (Figure 1B). In the cutaneous rpA, as predicted by previous reports^{6,7,9}, GPVI^{-/-} mice developed skin bleeding at the inflammatory reaction site, a bleeding phenotype that was seen neither in GPVI^{+/+} mice nor in GPVI^{+/-} mice, which expressed half of normal GPVI surface levels (Figure 1C-D). Taken together, these results are consistent with evidence that GPVI is dispensable for haemostasis in the inflamed brain and lungs^{6,16-18} but primarily involved in the prevention of bleeding in the rpA-inflamed skin. Notably, they further indicate that 50 % of normal GPVI surface levels are sufficient for haemostasis during the cutaneous rpA.

The ability of ACT017 to inhibit collagen/GPVI interactions and their functional consequences has been previously demonstrated in humans and in nonhuman primates¹⁴. However, it has not been tested in hGPVI mice. We thus verified the activity of ACT017 against GPVI from hGPVI mice. Like its murine precursor Fab 9O12¹, ACT017 added to whole blood from hGPVI mice caused a drastic reduction in platelet adhesion and aggregation onto collagen fibers under arterial and venous flow conditions (Figure 2A-B, Supplemental Movie). We next tested whether hGPVI mice treated with therapeutic (16 mg/kg) or higher doses of ACT017 (32 and 64 mg/kg) were sensitized to inflammation-induced bleeding during the cutaneous rpA. No bleeding occurred in ACT017-treated hGPVI mice, whatever

the dose of ACT017 used (data not shown and Figure 2C-D). There was no bleeding either when ACT017 at the highest dose tested (64 mg/kg) was given through a continuous infusion over the 4 hours of rpA (data not shown). The absence of bleeding in hGPVI mice treated with ACT017 was in contrast to the petechial bleeding observed in GPVI^{-/-} mice (Figure 1) and in platelet-depleted GPVI^{+/+} and hGPVI mice (Figures 1C-D and 2C-D), which is known to be a consequence of neutrophil recruitment⁷. Absence of bleeding in rpA-challenged hGPVI mice treated with ACT017 was not due to altered neutrophil recruitment as this was comparable to that in hGPVI mice (Figure 2E). Interestingly, the latter result indicates that ACT017 does not impact neutrophil recruitment, at least in this model. Importantly, ACT017 did not alter platelet recruitment to the inflamed skin either (Figure 2F). The latter result underscores a major difference between the impact of genetic deficiency in GPVI and that of GPVI blocking by ACT017. In fact, bleeding in rpA-challenged GPVI^{-/-} mice was previously shown to be associated with reduced platelet recruitment at the reaction site⁷.

Solid tumors represent another inflammatory situation in which platelets were shown to continuously prevent leukocyte-induced bleeding and recent data have suggested that GPVI could be central to this function¹⁹. Like in the cutaneous rpA, acute treatment of hGPVI mice bearing skin tumors with ACT017 did not cause tumor bleeding. The absence of effect of ACT017 on tumor vessel stability was in contrast to that of acute depletion of platelets, which caused tumor bleeding (Figure 2F).

In conclusion, in addition to confirming that GPVI is not required for inflammation-associated haemostasis in the brain and lungs, our results show that pharmacological blockade of GPVI by ACT017 does not impair GPVI-dependent inflammatory haemostasis. There are several non-exclusive reasons that could explain why pharmacological inhibition of GPVI by ACT017 does not impair the vasculoprotective recruitment of platelets during the cutaneous rpA. First, it was shown previously that GPVI can cooperate with other platelet receptors like

integrin alpha2beta1 to provide residual collagen-dependent platelet activation when its collagen binding site is blocked pharmacologically²⁰. Furthermore, while ACT017 blocks the interactions between GPVI and collagen, it remains unknown whether ACT017 has similar blocking effects towards the other ligands of GPVI. Besides collagen, fibrin(ogen) and a number of adhesive proteins of the vessel wall have been reported as GPVI ligands (e.g. laminin, fibronectin and vitronectin) and could thus provide redundant binding mechanisms. Moreover, were collagen to be one of the ligands supporting the adhesion of platelets to inflamed skin vessels, it is interesting to note that despite a drastic reduction in platelet adhesion and aggregation onto fibrillar collagen in the presence of ACT017 (Figure 2A-B, Supplemental Movie), residual platelet adhesion was observed at both arterial and venous blood flow. Considering that ACT017 has no effect on platelet recruitment during the cutaneous rpA, as well as previous results showing that individual platelets and platelet monolayers ensure haemostasis at sites of mild inflammatory vascular injury¹⁵, such residual interactions with collagen could be sufficient for inflammatory haemostasis. Previous studies have shown that platelets are particularly efficient in maintaining vascular integrity in inflamed organs, as platelet counts as low as 10% can support this function¹⁵. Consistent with this notion, GPVI^{+/-} mice with half of normal GPVI surface levels showed normal haemostasis during the cutaneous rpA (Figure 1C-D). All in all, our results indicate that the highly favorable safety profile of ACT017 suggested by previous results in bleeding time assays and by the absence of adverse bleeding events in the phase I clinical trial¹² also applies to inflammatory situations. Whether the safety profile of ACT017 still holds true when combining it with other drugs like recombinant tissue-type plasminogen activator remains to ascertain, but the absence of effect of ACT017 on platelet recruitment to the inflamed vasculature suggests there is a realistic chance for it to be maintained.

Disclosures

M. Jandrot-Perrus is the founder of Acticor Biotech. The other authors report no conflicts.

Acknowledgments

This work was supported by grants from INCA (2016-09/435/NI-KA), La Fondation ARC (PJA 20151203107), La Fondation pour la Recherche Médicale (grant #DPC20171138959), and by public grants overseen by the French National Research Agency (ANR) as part of the Investments for the Future program (PIA) under grant agreement No. ANR-18-RHUS-0001 (RHU Booster) and BPI (CMI2 project TherAVC2.0). Soumaya Jadoui was awarded a grant from la Société Française d'Hématologie.

References

1. Mangin PH, Tang CJ, Bourdon C, et al. A humanized glycoprotein VI (GPVI) mouse model to assess the antithrombotic efficacies of anti-GPVI agents. *J Pharmacol Exp Ther* 2012;341(1):156–163.
2. Dütting S, Bender M, Nieswandt B. Platelet GPVI: A target for antithrombotic therapy?! *Trends Pharmacol Sci* 2012;33(11):583–590.
3. Jiang P, Jandrot-Perrus M. New advances in treating thrombotic diseases: GPVI as a platelet drug target. *Drug Discovery Today* 2014;19(9):1471–1475.
4. Stegner D, Haining EJ, Nieswandt B. Targeting glycoprotein vi and the immunoreceptor tyrosine-based activation motif signaling pathway. *Arterioscler Thromb Vasc Biol* 2014;34(8):1615–1620.
5. Rayes J, Watson SP, Nieswandt B. Functional significance of the platelet immune receptors GPVI and CLEC-2. *J Clin Invest* 2019;129(1):12–23.
6. Rayes J, Jadoui S, Lax S, et al. The contribution of platelet glycoprotein receptors to inflammatory bleeding prevention is stimulus and organ dependent. *Haematologica* 2018;103(6):e256–e258.
7. Gros A, Syvannarath V, Lamrani L, et al. Single platelets seal neutrophil-induced vascular breaches via GPVI during immune-complex-mediated inflammation in mice. *Blood* 2015;126(8):1017–1026.
8. Hillgruber C, Pöppelmann B, Weishaupt C, et al. Blocking neutrophil diapedesis prevents hemorrhage during thrombocytopenia. *J Exp Med* 2015;212(8):1255–1266.
9. Boulaftali Y, Hess PR, Getz TM, et al. Platelet ITAM signaling is critical for vascular integrity in infammation. *J Clin Invest* 2013;123(2):908–916.
10. Maestrini I, Strbian D, Gautier S, et al. Higher neutrophil counts before thrombolysis for cerebral ischemia predict worse outcomes. *Neurology* 2015;85(16):1408–1416.
11. Michel JB, Martin-Ventura JL, Nicoletti A, Ho-Tin-Noé B. Pathology of human plaque vulnerability: Mechanisms and consequences of intraplaque haemorrhages. *Atherosclerosis* 2014;234(2):311–319.
12. Voors-Pette C, Lebozec K, Dogterom P, et al. Safety and Tolerability, Pharmacokinetics, and Pharmacodynamics of ACT017, an Antiplatelet GPVI (Glycoprotein VI) Fab: First-in-Human Healthy Volunteer Trial. *Arterioscler Thromb Vasc Biol* 2019;39(5):956–964.
13. Lecut C, Feeney LA, Kingsbury G, et al. Human platelet glycoprotein VI function is antagonized by monoclonal antibody-derived fab fragments. *J Thromb Haemost* 2003;1(12):2653–2662.
14. Lebozec K, Jandrot-Perrus M, Avenard G, Favre-Bulle O, Billiald P. Design, development and characterization of ACT017, a humanized Fab that blocks platelet's glycoprotein VI function without causing bleeding risks. *MAbs* 2017;9(6):945–958.
15. Ho-Tin-Noé B, Boulaftali Y, Camerer E. Platelets and vascular integrity: How platelets prevent bleeding in inflammation. *Blood* 2018;131(3):277–288.
16. Kraft P, Schuhmann MK, Fluri F, et al. Efficacy and safety of platelet glycoprotein receptor blockade in aged and comorbid mice with acute experimental stroke. *Stroke* 2015;46(12):3502–3506.
17. Kleinschnitz C, Pozgajova M, Pham M, Bendszus M, Nieswandt B, Stoll G. Targeting platelets in acute experimental stroke: Impact of glycoprotein Ib, VI, and IIb/IIIa blockade on infarct size, functional outcome, and intracranial bleeding. *Circulation* 2007;115(17):2323–2330.
18. Claushuis TAM, de Vos AF, Nieswandt B, et al. Platelet glycoprotein VI aids in local immunity during pneumonia-derived sepsis caused by gram-negative bacteria. *Blood* 2018;131(8):864–876.

19. Volz J, Mammadova-Bach E, Gil-Pulido J, et al. Inhibition of platelet GPVI induces intratumor hemorrhage and increases efficacy of chemotherapy in mice. *Blood* 2019;133(25):2696–2706.
20. Nieswandt B, Brakebusch C, Bergmeier W, et al. Glycoprotein VI but not $\alpha 2\beta 1$ integrin is essential for platelet interaction with collagen. *EMBO J* 2001;20(9):2120–2130.

Figure 1. Contribution of GPVI to inflammation-associated haemostasis. The contribution of GPVI to inflammation-associated haemostasis was determined in 3 different models of acute inflammation. **A.** Representative images of brain sections taken 24 hours after GPVI^{+/+}, GPVI^{-/-}, GPVI^{+/-}, and platelet-depleted mice were subjected to 90 min transient middle cerebral artery occlusion (tMCAO). Note that tMCAO caused bleeding only in platelet-depleted mice. The images are representative of n = 6 mice per group. **B.** Representative images of the bronchoalveolar lavage fluid from GPVI^{+/+}, GPVI^{-/-}, and platelet-depleted mice collected 24 hours after LPS inhalation. The images are representative of n = 8 mice per group. **C-D.** Effect of partial or complete glycoprotein VI deficiency on inflammatory bleeding during the cutaneous reverse passive Arthus reaction (rpA). **C.** Representative images of the skin of GPVI^{+/+}, GPVI^{+/-}, GPVI^{-/-}, and platelet-depleted mice after 4 hours of rpA. The images are representative of n = 7-10 mice per group. Bar = 500 μ m. **D.** Skin haemoglobin content after 4 hours of rpA. # indicates a significant difference ($p < 0.05$) from the rpA GPVI^{+/+} group, n=14-20 skin biopsies per group. Inset : Representative histogram of flow cytometry analysis of GPVI surface levels in GPVI^{+/+}, GPVI^{+/-}, and GPVI^{-/-} mice, as assessed using the JAQ1 antibody to mouse GPVI.

Figure 2. Impact of ACT017 on GPVI-dependent haemostasis. **A-B.** Citrated whole blood from hGPVI mice was labeled with the fluorochrome DiOC6, incubated or not with ACT017 (80 μ g/mL) for 10 min, and perfused at a wall shear rate of 1500 s^{-1} or 100 s^{-1} for 3 min over a collagen-coated surface. Bar = 50 μ m. **A.** Representative images of platelet coverage at the end of the perfusion. **B.** Mean surface areas covered by platelets calculated from 20 different fields taken with a 20 x objective along channels from 4 different runs (5 fields per run). **C.** Representative images of the skin of hGPVI mice treated or not with ACT017 (64 mg/kg) after 4 hours of rpA. The images are representative of n = 4-9 mice per group. Bar = 500 μ m. **D.** Skin haemoglobin content after 4 hours of rpA. # indicates a significant difference ($p < 0.05$) from the rpA hGPVI group, n=6-18 skin biopsies per group. **E-F.** Skin myeloperoxidase (**E**) and platelet factor 4 (PF4) (**F**) content after 2 hours of rpA, as assessed by ELISA. n=12 skin biopsies per group.