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 time. 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 tissues. 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 stroke and contributes to intraplaque hemorrhage, 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 volunteers and has just entered a phase II trial in stroke patients (Acute Ischemic Stroke Interventional Study [ACTIMIS], clinicaltrials.gov. Identifier: 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 on collagen surfaces under arterial flow conditions. The inhibitory action of ACT017 occurs without causing thrombocytopenia or depletion of GPVI, and is not associated with spontaneous bleeding events or increased bleeding time. 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 inflammation. Here, using the cutaneous reverse passive Arthus reaction (pA) as a model situation where GPVI plays a major role in inflammatory hemostasis, 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, there was no cerebral hemorrhage in any of the GPVI-/- mice subjected to transient (90 minutes) middle cerebral artery occlusion (Figure 1A). In contrast, cerebral hemorrhage 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 Pseudomonas aeruginosa endotoxin either (Figure 1B). In the cutaneous pA, as predicted by previous reports, 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 and D). Taken together, these results are consistent with evidence that GPVI is dispensable for hemostasis in the inflamed brain and lungs but primarily involved in the prevention of bleeding in the pA-depleted mouse. Notably, they further indicate that more than 50% of normal GPVI surface levels are sufficient for hemostasis during the cutaneous pA.

The ability of ACT017 to inhibit collagen/GPVI interactions and their functional consequences has been previously demonstrated in humans and in nonhuman pri-
Figure 1. Contribution of glycoprotein VI to inflammation-associated hemostasis. The contribution of glycoprotein VI (GPVI) to inflammation-associated hemostasis was determined in three different models of acute inflammation. (A) Representative images of brain sections taken 24 hours after GPVI+/+, GPVI−/−, and platelet-depleted mice were subjected to 90 minutes 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 lipopolysaccharide inhalation. The images are representative of n=8 mice per group. (C and D) Effect of partial or complete GPVI deficiency on inflammatory bleeding during the cutaneous reverse passive Arthus reaction (rpA). (C) Representative images of the skin of GPVI+/+, GPVI−/−, and platelet-depleted mice after 4 hours of rpA. The images are representative of n=7-10 mice per group. Bar =500 μm. (D) Skin hemoglobin 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 representative mouse GPVI.
aggregation onto fibrillar collagen in the presence of ACT017 (Figure 2A and B; Online Supplementary Movie), residual platelet adhesion was observed at both arterial and venous blood flow. Considering that ACT017 has no effect on platelet recruitment during cutaneous rpA and that previous results have shown that individual platelets and platelet monolayers ensure hemostasis at sites of mild inflammatory vascular injury, such residual interactions with collagen could be sufficient for inflammatory hemostasis. Previous studies have also shown that platelets are particularly efficient in maintaining vascular integrity in inflamed organs, as platelet counts as low as 10% can support this function. Consistent with this notion, Gpvi<sup>−/−</sup> mice with half of normal GPVI surface levels showed normal hemostasis during the cutaneous rpA (Figure 1C and 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 trial 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.  


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References  