GPVI inhibition by glenzocimab synergistically inhibits atherosclerotic plaque-induced platelet activation when combined with conventional dual antiplatelet therapy

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Introduction: Aspirin and a potent platelet P2Y12 inhibitor, such as prasugrel or ticagrelor, are not always sufficient to prevent thrombus formation in patients with ST-elevation MI (STEMI), leading to "slow flow" or "no reflow" effects after stenting. GPIIb/IIIa inhibitors, such as eptifibatide, may help in this setting, but are not used routinely due to their bleeding risk. GPVI has critical roles in thrombosis and a minimal role in haemostasis. Here we tested whether depletion of GPVI has effects on thrombus formation after MI in an animal model and investigated the effects of a novel platelet GPVI inhibitor, glenzocimab (a Fab fragment of a monoclonal antibody), on platelet activation and thrombus formation when combined with aspirin and ticagrelor.

Methods: We used intravital microscopy in a murine model of ST-elevation myocardial infarction and ischaemia-reperfusion injury to investigate microvascular thrombosis. We investigated the antithrombotic effects of adding glenzocimab (previously known as ACT017) to blood from healthy donors and 20 patients with ACS treated with aspirin and ticagrelor. We compared the effect of glenzocimab with the GPIIb/IIIa inhibitor eptifibatide ex-vivo. We stimulated platelets with collagen and atherosclerotic plaque material that was sourced from patients undergoing carotid endarterectomy. We investigated effects on platelet aggregation, spreading, signalling, adhesion, thrombin generation, thrombus formation and clot stability ex vivo.

Results: Genetic depletion of GPVI in an animal model of myocardial infarction reduced microvascular thrombosis. Ex vivo, aspirin and ticagrelor partially inhibited atherosclerotic plaque-induced platelet aggregation (assessed by multiple electrode aggregometry) by 48% compared to control (34±3 vs. 65±4 U; $P<0.001$; Figure 1). Atherosclerotic plaque-induced platelet aggregation, adhesion, secretion and activation were critically dependent on platelet GPVI activation and were potently inhibited by glenzocimab. Glenzocimab alone reduced atherosclerotic plaque-induced platelet aggregation by 75% compared to control (16±4 vs. 65±4 U; $P<0.001$; Figure 1) and by over 95% when combined with aspirin and ticagrelor (3±1 vs 65±4 U; $P<0.001$; Figure 1). Furthermore, glenzocimab provided multiple synergistic antithrombotic effects when added to the blood of aspirin and ticagrelor-treated patients with ACS ex vivo. Glenzocimab and the GPIIb/IIIa inhibitor, eptifibatide, had many similar antithrombotic effects but glenzocimab had less effect on mechanisms of general haemostasis compared to eptifibatide, as assessed by ROTEM (Figure 2).

Conclusions: The addition of glenzocimab to aspirin and ticagrelor provides synergistic inhibition of multiple critical mechanisms of atherothrombosis. Glenzocimab and the GPIIb/IIIa inhibitor, eptifibatide, share many similar antithrombotic effects, although glenzocimab has less impact on mechanisms involved in haemostasis compared to eptifibatide.

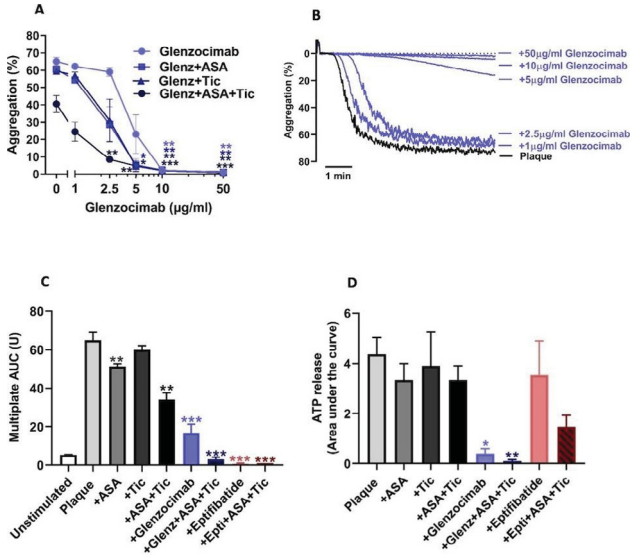

Figure 1. Effect of glenzocimab on atherosclerotic plaque-induced platelet responses, compared to aspirin (ASA), ticagrelor (Tic) and eptifibatide (Epti) *ex vivo*. Platelet aggregation assessed by light transmission aggregometry (A) with representative traces (B) and multiple electrode aggregometry (C). Platelet secretion assessed by Chrono Lume assay (D).

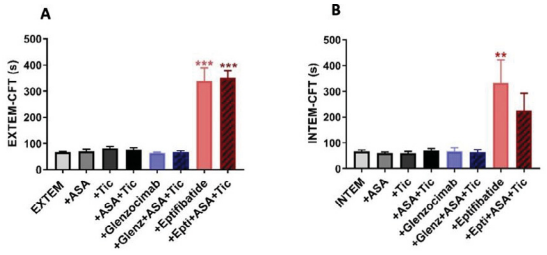

Figure 2. Mechanisms of haemostasis as determined by ROTEM thromboelastography. Glenzocimab compared to aspirin (ASA), ticagrelor (Tic) and eptifibatide (Epti). Clot formation time (CFT) reflects the time taken for platelets to aggregate and for fibrin to form in response to activation of the extrinsic (EXTEM) and intrinsic (INTEM) coagulation system. Values over 147s (EXTEM) and 111s (INTEM) are associated with major bleeding.