Amplified inhibition of atherosclerotic plaque-induced platelet activation by glenzocimab with dual antiplatelet therapy JTH

Alenazy, Fawaz; Harbi, Maan; Kavanagh, Dean; Price, Joshua; Brady, Paul; Hargreaves, Oscar; Harrison, Paul; Slater, Alexandre; Tiwari, Alok; Nicolson, Pip; Connolly, Derek; Kirchhof, Paulus; Kalia, Neena; Jandrot-Perrus, Martine; Mangin, Pierre H; Watson, Steve; Thomas, Mark R.

DOI:
10.1016/j.jtha.2023.07.018

Received: 22 January 2023 | Revised: 23 June 2023 | Accepted: 16 July 2023

https://doi.org/10.1016/j.jtha.2023.07.018

ORIGINAL ARTICLE

Amplified inhibition of atherosclerotic plaque-induced platelet activation by glenzocimab with dual antiplatelet therapy

Fawaz O. Alenazy[1,2] | Maan H. Harbi[1,3] | Dean P. Kavanagh[1] | Joshua Price[1] | Paul Brady[1,4] | Oscar Hargreaves[1] | Paul Harrison[5] | Alexandre Slater[1] | Alok Tiwari[6] | Phillip L. R. Nicolson[1] | Derek L. Connolly[4] | Paulus Kirchhof[1,7,8] | Neena Kalia[1] | Martine Jandrot-Perrus[9] | Pierre H. Mangin[10] | Steve P. Watson[1,11] | Mark R. Thomas[1,4,12]

[1]Institute of Cardiovascular Sciences, University of Birmingham, Birmingham, United Kingdom

[2]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Sakaka, Saudi Arabia

[3]Pharmacology and Toxicology Department, College of Pharmacy, Umm Al-Qura University, Makkah, Saudi Arabia

[4]Department of Cardiology, Sandwell and West Birmingham Hospitals National Health Service (NHS) Trust, Birmingham, United Kingdom

[5]Institute of Inflammation and Ageing, University of Birmingham, Birmingham, United Kingdom

[6]Department of Vascular Surgery, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom

[7]Department of Cardiology, University Heart and Vascular Center (UKE) Hamburg, Hamburg, Germany

[8]German Center for Cardiovascular Research (DZHK), Partner Site Hamburg/Kiel/Lübeck, Germany

[9]UMR_S1148, INSERM, Paris, France

[10]UMR_S1255, INSERM, Etablissement Francais du Sang-Alsace, Strasbourg, France

[11]Centre of Membrane Proteins and Receptors (COMPARE), The Universities of Birmingham and Nottingham, The Midlands, United Kingdom

Abstract

Background: Aspirin and platelet $P2Y_{12}$ inhibitors, such as ticagrelor, suboptimally inhibit microvascular thrombosis during ST-elevation myocardial infarction. Glycoprotein (GP) IIb/IIIa inhibitors may further inhibit this but cause excessive bleeding.

Objectives: We investigated whether combination of glenzocimab, a GPVI inhibitor, with aspirin and ticagrelor provides additional antithrombotic effects, as GPVI has a critical role in atherothrombosis but minimal involvement in hemostasis.

Methods: We investigated the effects of glenzocimab (monoclonal antibody Fab fragment) using blood from healthy donors and patients with acute coronary syndrome treated with aspirin and ticagrelor. Platelets were stimulated with multiple agonists, including atherosclerotic plaque, from patients undergoing carotid endarterectomy.

Results: Aspirin and ticagrelor partially inhibited atherosclerotic plaque-induced platelet aggregation by 48% compared with control (34 ± 3 vs 65 ± 4 U; $P < .001$). Plaque-induced platelet aggregation, adhesion, secretion, and activation were critically dependent on GPVI activation. Glenzocimab alone reduced plaque-induced aggregation by 75% compared with control (16 ± 4 vs 65 ± 4 U; $P < .001$) and by >95% when combined with aspirin and ticagrelor (3 ± 1 vs 65 ± 4 U; $P < .001$). Glenzocimab reduced platelet aggregation, adhesion, and thrombin generation when added to blood of aspirin- and ticagrelor-treated patients with acute coronary syndrome. Glenzocimab shared several antithrombotic effects with the GPIIb/IIIa inhibitor eptifibatide with less effect on general hemostasis assessed by rotational thromboelastometry. In a murine intravital model of ST-elevation myocardial infarction, genetic depletion of GPVI reduced microvascular thrombosis.

Conclusion: Addition of glenzocimab to aspirin and ticagrelor enhances platelet inhibition via multiple mechanisms of atherothrombosis. Compared with a GPIIb/IIIa

Manuscript handled by: Kellie Machlus

Final decision: Kellie Machlus, 17 July 2023

[12]Department of Cardiology, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom

Correspondence
Mark R. Thomas, Institute of Cardiovascular Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.
Email: markthomas@doctors.org.uk

Funding information
This work was funded by a Wellcome Trust ISSF Accelerator Fellowship and Academy of Medical Sciences Clinical Lecturer Starter Grant awarded to M.R.T. (SGL018\1114) and a scholarship awarded to F.O.A. by the Royal Embassy of Saudi Arabia. Glenzocimab was provided as a gift from Acticor Biotech. This research was also partly funded by a Wellcome Trust Grant (204951/Z/16/Z) to S.P.W. and the British Heart Foundation (BHF: CH03/003).

inhibitor, glenzocimab shares multiple antithrombotic effects, with less inhibition of mechanisms involved in general hemostasis.

KEYWORDS
acute coronary syndromes, antiplatelet therapy, atherosclerotic plaque, GPVI, pharmacology, platelets thrombosis, translational studies

1 | INTRODUCTION

Despite contemporary dual antiplatelet therapy, consisting of aspirin and a potent platelet $P2Y_{12}$ inhibitor, such as ticagrelor, up to 40% of patients with ST-elevation myocardial infarction (STEMI) develop microvascular obstruction [1]. Microvascular obstruction leads to increased myocardial infarct size, and patients have a particularly high risk of subsequent major adverse cardiovascular events of approximately 20% in the first year [1]. Embolization of thrombus and atherosclerotic plaque material is thought to play an important role in the pathophysiology of microvascular obstruction, but this process is multifactorial and still incompletely understood [2,3]. There is limited evidence that platelet glycoprotein (GP) IIb/IIIa inhibitors, such as eptifibatide, can reduce microvascular obstruction if administered directly into the coronary vasculature [4]. However, they cannot be used routinely because of their significant effect on general hemostasis and are associated with an increased incidence of major bleeding [5]. This presents an unmet clinical need for novel antiplatelet strategies that provide more antithrombotic potency than aspirin and potent $P2Y_{12}$ inhibitors but have less impact on hemostasis than the addition of GPIIb/IIIa inhibitors, such as eptifibatide.

Inhibition of platelet GPVI presents a promising antithrombotic strategy that blocks multiple critical mechanisms of atherothrombosis while leaving general hemostasis intact [6]. GPVI is the main platelet activation receptor for collagen, which is one of the most prothrombotic components of atherosclerotic plaque that is exposed after plaque rupture [7]. Recent studies have demonstrated that GPVI is also a receptor for fibrin and has major roles in thrombus growth and stability [8,9]. In animal models, inhibition of platelet GPVI improves microperfusion following obstruction of a coronary artery, resulting in a reduction in myocardial infarct size [10]. Despite the major roles of GPVI in several critical aspects of thrombosis, GPVI deficiency is generally associated with minimal impact on hemostasis in animal models and does not affect tail bleeding time in mice [11,12]. GPVI deficiency is very rare in humans and again only appears to have a minimal impact on hemostasis [13]. In particular, it is clear that GPVI deficiency does not cause a major bleeding defect like GPIIb/IIIa deficiency (Glanzmann thrombasthenia) [14]. Furthermore, GPVI is only present on platelets, thereby reducing the risk of GPVI inhibitors having off-target effects.

Glenzocimab (previously known as ACT017) is a novel humanized antibody fragment that blocks platelet GPVI and, therefore, potently inhibits collagen-induced platelet aggregation [15,16]. Emerging evidence also shows that glenzocimab is able to cause platelets in a preformed thrombus to disaggregate [17]. A phase I clinical trial published in 2019 demonstrated that a single dose of glenzocimab has a half-life of approximately 12 to 24 hours and was safe, well-tolerated, and had no effect on hemostasis, as assessed by bleeding time [15]. In a phase Ib study of fibrinolysis-treated patients with stroke, glenzocimab had a favorable safety profile with no overt effect on risk of intracranial

Essentials

- The glycoprotein (GP) VI inhibitor glenzocimab potently inhibits atherosclerotic plaque-induced platelet activation.
- Combination of glenzocimab with aspirin and ticagrelor amplifies multiple antithrombotic effects.
- Glenzocimab shares many antithrombotic effects with GPIIb/IIIa inhibitors.
- GPVI inhibition appears to have less impact on mechanisms of hemostasis than GPIIb/IIIa inhibitors.

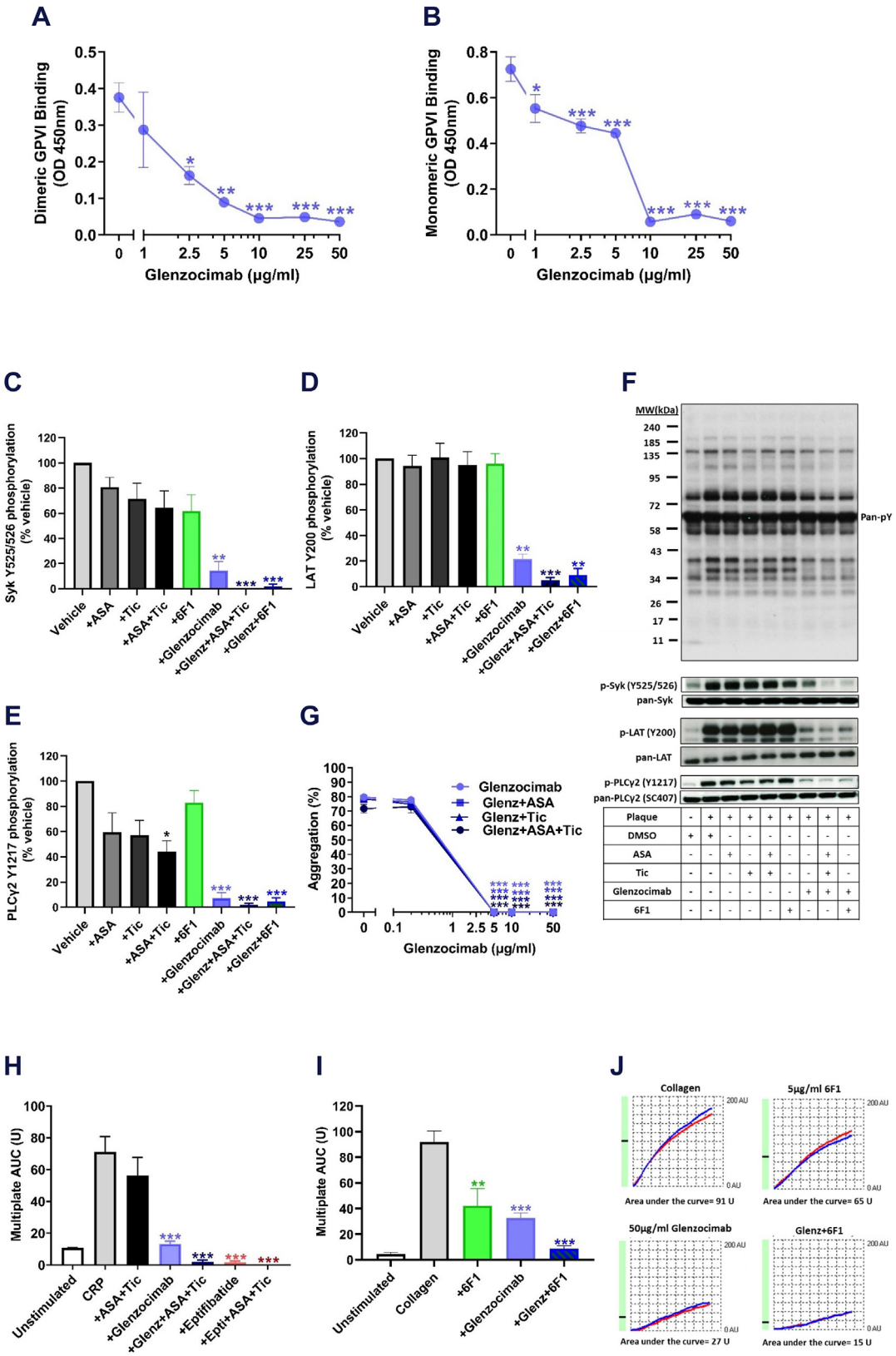

FIGURE 1 Inhibition of platelet glycoprotein (GP) VI signaling and platelet aggregation in response to atherosclerotic plaque, collagen, and collagen-related peptide. Blood was sampled from healthy volunteers, and agonists and inhibitors were added *ex vivo*. (A) Dose-dependent inhibition of dimeric GPVI (2.5 µg/mL) binding to 70 µg/mL atherosclerotic plaque by glenzocimab 1-50 µg/mL. (B) Dose-dependent inhibition of monomeric GPVI binding (2.5 µg/mL) to 70 µg/mL atherosclerotic plaque by glenzocimab 1-50 µg/mL (C) Glenzocimab 50 µg/mL alone significantly decreases 70 µg/mL atherosclerotic plaque-mediated Syk (Y525/526) phosphorylation and the addition of antiplatelet agents

hemorrhage or other forms of major bleeding [18]. On the basis of this study, a 1 g dose of glenzocimab was chosen as the optimal dose to clinically investigate and a phase I study previously showed this dose results in a C_{max} of 59.9 μg/mL [15].

Previous studies have demonstrated that individual antagonists of platelet receptors $P2Y_{12}$, GPVI, α2β1, and GPIb can inhibit collagen and atherosclerotic plaque to varying degrees [7,17,19–21] and we aimed to determine the additional effects of a GPVI inhibitor compared to other inhibitors. In a clinical trial of patients with STEMI, a specific GPVI inhibitor could currently only be investigated in combination with aspirin and $P2Y_{12}$ inhibitors, as it would be considered unethical to withhold these well-established treatments until glenzocimab has been established to also provide similar benefits in patients. Furthermore, as glenzocimab is administered intravenously, coadministration with aspirin and a potent $P2Y_{12}$ inhibitor would also facilitate long-term treatment with these medications. It has not previously been shown whether the GPVI inhibitor glenzocimab blocks atherosclerotic plaque-induced platelet responses or whether it provides additional effects compared to aspirin and a potent $P2Y_{12}$ inhibitor. We therefore investigated whether glenzocimab provides additional antithrombotic effects compared to aspirin and ticagrelor, which are used routinely for the treatment of STEMI.

2 | METHODS

Detailed materials and methods are described in the Supplementary Appendix.

2.1 | Patient samples

Aspirin, ticagrelor, glenzocimab, and eptifibatide were added to the blood of healthy volunteers *ex vivo*. Blood was also sampled from 20 aspirin- and ticagrelor-treated patients with acute coronary syndrome (ACS) and either glenzocimab or eptifibatide were added *ex vivo*. We assessed the effects of glenzocimab using dose-response curves. In experiments with a single concentration of glenzocimab, we used 50 μg/mL glenzocimab as this is representative of the C_{max} (59.9 μg/mL) after a 1 g dose [17]. Atherosclerotic plaque was obtained from 10 patients with symptomatic carotid artery stenosis undergoing carotid endarterectomy, then homogenized and pooled. All experiments involving human subjects were performed in accordance with the Declaration of Helsinki and Good Clinical Practice and approved by the National Health Service Research and Ethics Committees (North West – Haydock Research Ethics Committee 20/NW/0001 and West Midlands – South Birmingham Research Ethics Committee 18/WM/0386). Use of blood from healthy volunteers was approved by the University of Birmingham Ethics Review (ERN_11-0175).

2.2 | Assessment of platelet function

Platelet aggregation was assessed by light transmission aggregometry and multiple electrode aggregometry. Platelet adhesion and aggregation under flow conditions were assessed using microfluidics and an automated imaging system (EVOS, Thermo Fisher Scientific). Platelet spreading was assessed using an optical assay. Platelet signaling was assessed by Western blotting. Thrombin generation was assessed using a calibrated automated thrombogram (Stago). Clot viscoelastic properties were assessed using rotational thromboelastometry (ROTEM).

2.3 | Experimental animals and study design

All experiments were performed in accordance with UK laws with UK Home Office approval under PPL P0E98D513 and 70/8576. $GPVI^{-/-}$ mice were compared with wild-type (WT) using intravital imaging of a myocardial ischemia-reperfusion model as previously described (described in more detail in the Supplementary Appendix) [22].

2.4 | Statistical analysis

Continuous variables are presented as means ± SEM. Differences in continuous variables between groups were assessed either by 1- or 2-way analysis of variance (ANOVA) as appropriate with Dunnett's correction for multiple comparison. If ANOVA was not computable due to one or more missing values, then a linear mixed model analysis was utilised in its place. A 2-tailed *P* value of less than .05 was considered significant. All statistical analyses were performed using GraphPad Prism (version 9).

(ASA) 30 μM and ticagrelor 1 μM or 6F1 10 μg/mL on top of glenzocimab completely inhibit Syk (Y525/526) phosphorylation. (D) Glenzocimab 50 μg/mL alone blocks atherosclerotic plaque-induced linker for activation of T cells (LAT) (Y200) phosphorylation, and its inhibitory effect is amplified by ASA 30 μM and ticagrelor 1 μM or 6F1 10 μg/mL. (E) Glenzocimab 50 μg/mL inhibits atherosclerotic plaque-induced PLCγ2 (Y1217) phosphorylation and completely blocks tyrosine phosphorylation upon combination with ASA and ticagrelor, which on their own partially reduced phosphorylation. (F) Representative western blot images showing plaque activates platelets via a GPVI signaling-dependent pathway, which is significantly inhibited by the novel GPVI inhibitor, glenzocimab. (G) Glenzocimab at 5 μg/mL fully blocks 3 μg/mL collagen-related peptide (CRP)-induced platelet aggregation in platelet-rich plasma (PRP) in light transmission aggregometry with no inhibitory effect observed for ASA 30 μM and ticagrelor 1 μM. (H) Glenzocimab 50 μg/mL potently inhibited 3 μg/mL CRP in whole blood in multiple electrode aggregometry, leaving only slight residual platelet aggregation ie, fully blocked by the addition of ASA 30 μM and ticagrelor 1 μM. Eptifibatide 9 μM entirely inhibits CRP-stimulated platelet aggregation. (I) Glenzocimab 50 μg/mL incompletely inhibits collagen-stimulated platelet aggregation in hirudinized whole blood, indicating involvement of the other collagen receptor (integrin α2β1), which was confirmed by full blockade with the addition of 6F1 10 μg/mL. (J) Representative multiple electrode aggregometry tracings for blockade of collagen-mediated platelet aggregation by glenzocimab and 6F1. Results are expressed as mean ± SEM (*n* = 3-4). The effect of treatment groups was compared with the uninhibited sample (control) using 1- or 2-way ANOVA followed by Dunnett's correction for multiple comparisons (**P* < .05, ***P* < .01, and ****P* < .001). AUC, area under the curve.

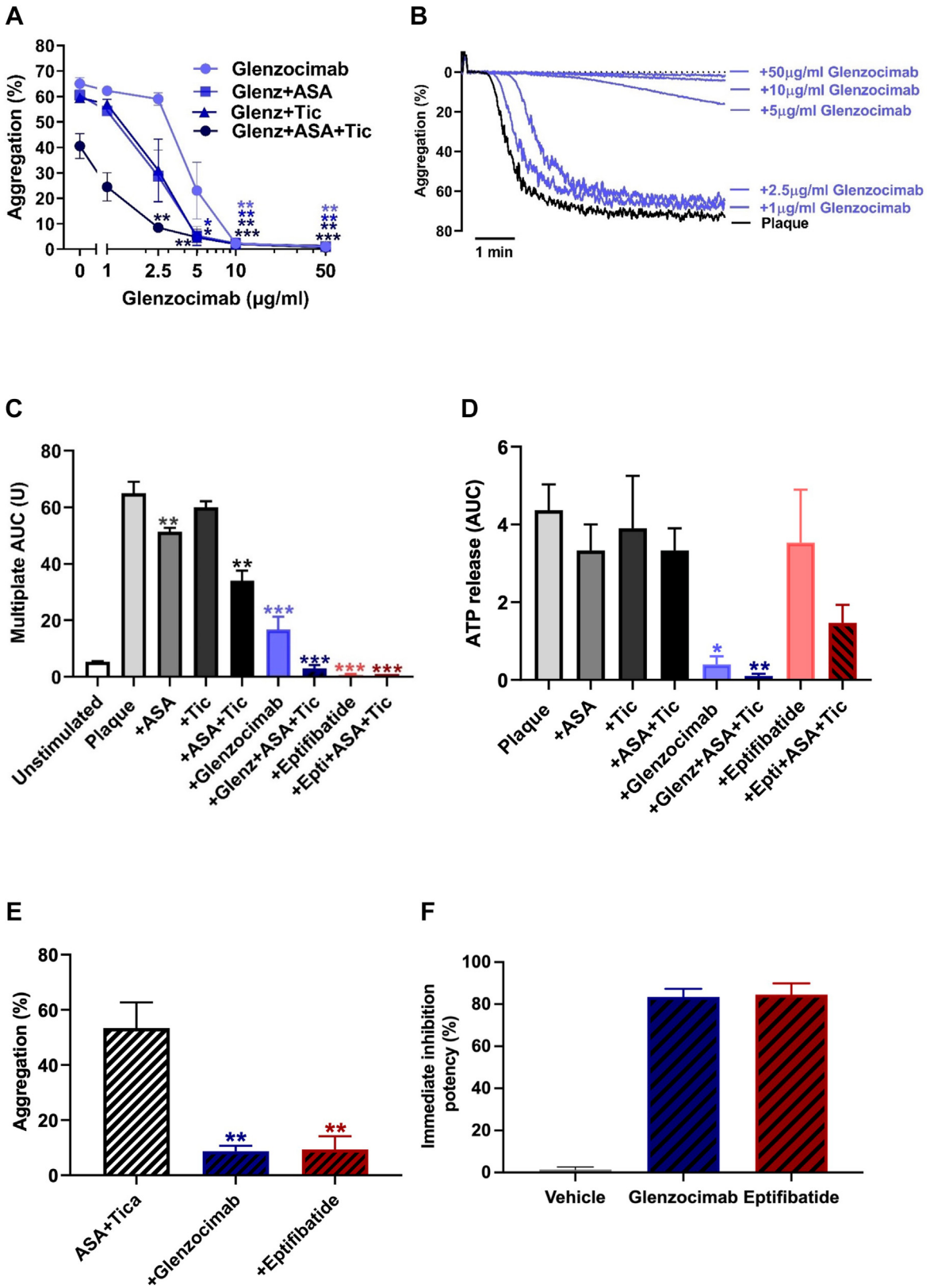

FIGURE 2 Glenzocimab inhibits atherosclerotic plaque-mediated platelet aggregation and ATP secretion. (A) Glenzocimab (1-50 μg/mL) dose-dependently inhibits atherosclerotic plaque-induced platelet aggregation and is amplified by the addition of aspirin 30 μM and ticagrelor 1 μM in PRP assessed by light transmission aggregometry (LTA). (B) Representative tracings of dose-dependent inhibition of atherosclerotic plaque-induced platelet aggregation by glenzocimab. (C) Glenzocimab 50 μg/mL provides further inhibition of atherosclerotic plaque-induced platelet aggregation when combined with ASA 30 μM and ticagrelor 1 μM, which is similar to eptifibatide 9 μM in whole blood aggregation assessed by multiple electrode aggregometry. (D) Glenzocimab 50 μg/mL potently blocks atherosclerotic plaque-stimulated platelet ATP

3 | RESULTS

3.1 | Glenzocimab binds to both monomeric and dimeric GPVI and abolishes platelet GPVI signaling in response to atherosclerotic plaque

GPVI is expressed on platelet membranes in monomeric and dimeric forms, which can differ in their affinity for GPVI ligands [23]. ELISA demonstrated that atherosclerotic plaque material bound to both the monomeric and dimeric forms of GPVI (Supplementary Figure S1A, B). Atherosclerotic plaque strongly induced tyrosine phosphorylation of Syk, linker for activation of T cells, and PLCγ2 (Figure 1C–E), consistent with activation of GPVI, which was sustained for at least 1 hour (Supplementary Figure S2A–E). Glenzocimab inhibited the binding of atherosclerotic plaque to both monomeric and dimeric GPVI (Figure 1A, B) and abolished GPVI activation and downstream signaling of Syk, linker for activation of T cells, and PLCγ2 in response to atherosclerotic plaque material (Figure 1C–F). We also used the specific GPVI agonist collagen-related peptide (CRP) to confirm that glenzocimab fully inhibits GPVI-mediated platelet responses (Figure 1G, H).

In contrast, aspirin and ticagrelor did not significantly reduce activation of GPVI and its downstream signaling in response to atherosclerotic plaque material, apart from partial inhibition of PLCγ2 phosphorylation (Figure 1C–F). In keeping with this, aspirin and ticagrelor also only had minimal effect on platelet aggregation induced by the specific GPVI agonist CRP (Figure 1G, H).

We then investigated whether atherosclerotic plaque also activated another major collagen receptor, α2β1, using 6F1, a monoclonal antibody that specifically inhibits α2β1. 6F1 had no effect on atherosclerotic plaque-induced platelet signaling or aggregation, indicating that these were not mediated by α2β1 (Figure 1C–J). Horm collagen is a commercial preparation of a mixture of nonhuman collagens that is commonly used to investigate platelet function. Glenzocimab did not completely inhibit Horm collagen-induced platelet responses (Figure 1I). The residual response could be fully inhibited by the addition of 6F1, indicating that response to Horm collagen is both GPVI- and α2β1-dependent.

3.2 | Glenzocimab blocks atherosclerotic plaque-induced platelet aggregation and secretion

Atherosclerotic plaque-induced platelet aggregation was assessed by both light transmission aggregometry and multiple electrode aggregometry (Figure 2A–F). The combination of aspirin and the potent $P2Y_{12}$ inhibitor ticagrelor partially inhibited atherosclerotic plaque-induced platelet aggregation by ~25% to 40% (Figure 2A, C) when used at therapeutic concentrations (C_{max} of aspirin is approximately 20 μM [24], and C_{max} of ticagrelor is approximately 1 μM [25]) that fully inhibited their specific target pathways (Supplementary Figure S3). We investigated the effect of 1 to 50 μg/mL of glenzocimab, as this corresponds with the C_{max} of ~60 μg/mL when glenzocimab is administered to humans at an optimal dose [15]. At 10 to 50 μg/mL concentrations, glenzocimab provided near-complete inhibition of atherosclerotic plaque-induced platelet aggregation, which was entirely inhibited by further addition of aspirin and ticagrelor (Figure 2A, C). Eptifibatide alone also provided complete inhibition of plaque-induced platelet aggregation, and the combination of aspirin, ticagrelor, and glenzocimab provided similar levels of inhibition of platelet aggregation as the combination of aspirin, ticagrelor, and eptifibatide (Figure 2A, C). Glenzocimab provided potent inhibition of platelet secretion, assessed by adenoine triphosphate release, to a greater extent than achieved by aspirin, ticagrelor, or eptifibatide, either on their own or in combination (Figure 2D). We also investigated whether these drugs were able to cause platelet disaggregation after aggregation had already commenced following exposure to atherosclerotic plaque material. Both glenzocimab and eptifibatide caused disaggregation when added to blood that had been pretreated with aspirin and ticagrelor and then exposed to atherosclerotic plaque (Figure 2E, F). Since glenzocimab potently inhibited platelet activation, we next investigated whether it blocked platelet adhesion.

3.3 | Glenzocimab blocks platelet activation and spreading following adhesion to atherosclerotic plaque material

Under flow conditions, the combination of aspirin and ticagrelor caused partial inhibition of platelet adhesion to atherosclerotic plaque material and P-selectin expression (Figure 3A–D). Glenzocimab and the combined use of aspirin, ticagrelor, and glenzocimab again caused near-complete inhibition of platelet adhesion to atherosclerotic plaque material and P-selectin expression, which was comparable to the effect of eptifibatide (Figure 3A–D). Under static conditions, the combination of aspirin and ticagrelor had no significant effect on platelet adhesion to atherosclerotic plaque (Figure 3E–G). The combination of glenzocimab, aspirin, and ticagrelor, in contrast, potently inhibited platelet adhesion to atherosclerotic plaque, and eptifibatide only reduced platelet spreading but not adhesion (Figure 3E–G).

secretion, while conventional antiplatelet agents (ASA 30 μM, ticagrelor 1 μM, and eptifibatide 9 μM) only produce significant effects when combined. (E) Glenzocimab 50 μg/mL and eptifibatide 9 μM similarly reverse aggregation of ASA and ticagrelor pretreated platelets after stimulation with atherosclerotic plaque in PRP assessed by LTA. (F) The disaggregation potency of glenzocimab and eptifibatide on atherosclerotic plaque-stimulated aggregation of ASA and ticagrelor pretreated platelets. The results are expressed as mean ± SEM (n = 3-4). The effect of treatment groups was compared with the uninhibited sample (control) using 1- or 2-way ANOVA followed by Dunnett's correction for multiple comparisons (*P < .05, **P < .01, and ***P < .001). ATP, adenoine triphosphate; AUC, area under the curve; PRP, platelet-rich plasma.

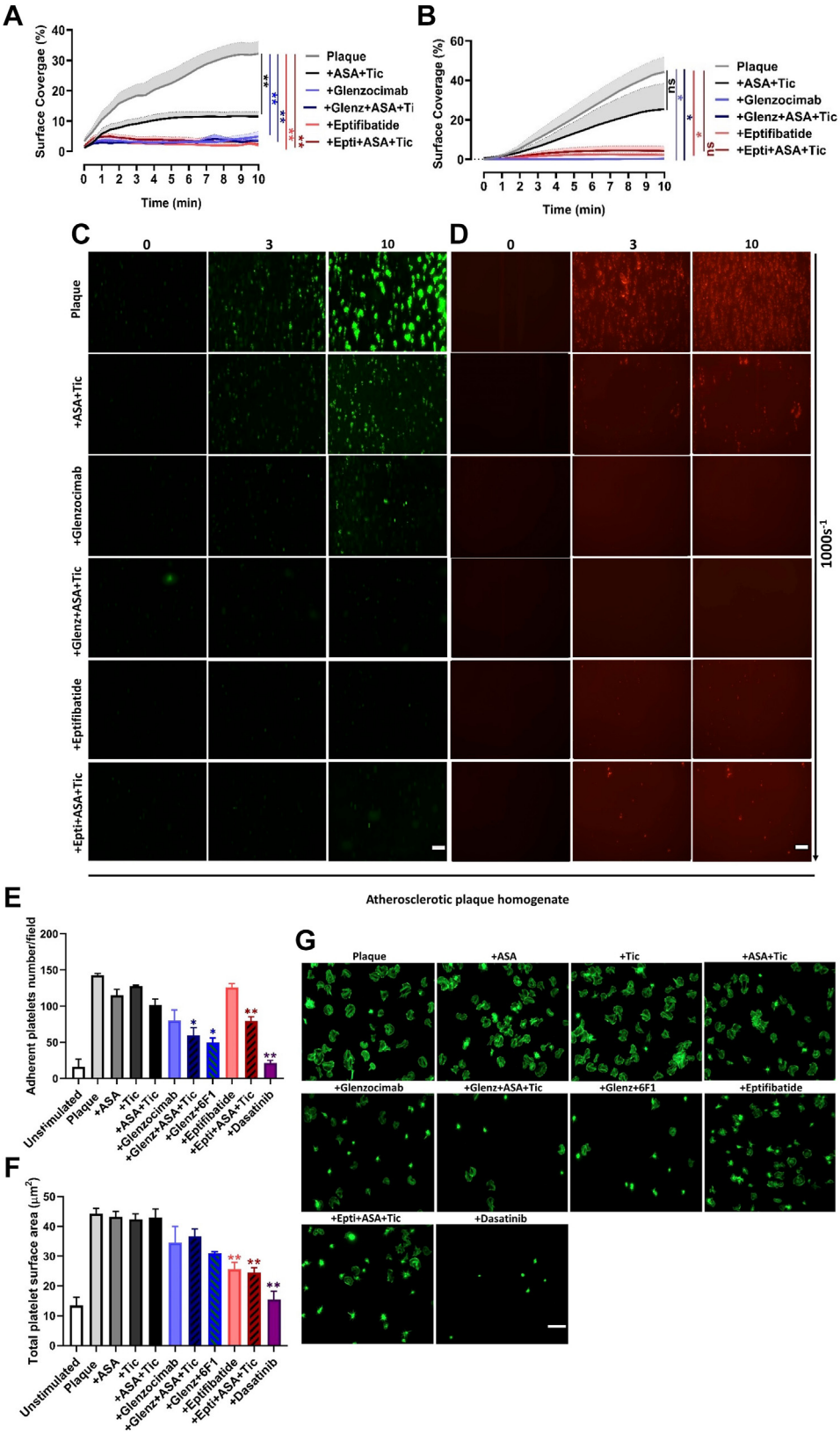

FIGURE 3 Glenzocimab inhibits platelet-thrombus formation and P-selectin expression on atherosclerotic plaque in a microfluidic chamber at arterial shear rates and also reduces platelet adhesion to atherosclerotic plaque under static conditions when combined with ASA and ticagrelor. (A) Glenzocimab 50 μg/mL and eptifibatide 9 μM profoundly block platelet-thrombus formation and platelet adhesion on 1 mg/mL atherosclerotic plaque in flowing blood at 1000 s^{-1} as reflected by surface coverage percentage while dual treatment with ASA 30 μM and ticagrelor 1 μM partially reduced platelet adhesion. (B) Glenzocimab 50 μg/mL entirely inhibits P-selectin expression on 1 mg/mL

3.4 | Glenzocimab inhibits the procoagulant function of platelets, including phosphatidylserine exposure, thrombin generation, and fibrin-mediated platelet aggregation

GPVI is involved in a complex amplification loop between platelets and the coagulation system, particularly driven by platelet activation driven by fibrin, as well as collagen, and atherosclerotic plaque material [8,9,21,26]. We therefore next investigated whether glenzocimab was able to inhibit fibrin-induced platelet responses, thereby blocking subsequent phosphatidylserine exposure and thrombin generation, which further drive fibrin formation.

Fibrin induced ~45% platelet aggregation, which was reduced to approximately 20% by aspirin and ticagrelor (Figure 4A). Glenzocimab provided potent inhibition, reducing platelet aggregation to 7%, which was similar to eptifibatide (Figure 4B). In a microfluidic model of platelet-thrombus formation on collagen under arterial flow conditions, aspirin, ticagrelor, and eptifibatide had no significant effect on platelet procoagulant status, as assessed by Annexin V expression in response to collagen exposure (Figure 4C, D). In contrast, this was potently inhibited by glenzocimab, with near-complete inhibition when glenzocimab was used in combination with aspirin and ticagrelor (Figure 4C, D). We next assessed tissue factor-induced platelet thrombin generation using a calibrated automated thrombogram. This showed no effect of aspirin and ticagrelor on thrombin generation in platelet-rich plasma, whereas glenzocimab and eptifibatide both inhibited peak thrombin generation by >50% (Figure 4E, F).

3.5 | *Ex vivo* addition of glenzocimab provides amplified antithrombotic effects in blood from aspirin- and ticagrelor-treated patients with ACS

We next evaluated the effect of the addition of glenzocimab and eptifibatide *ex vivo* on platelet function when added to blood samples from aspirin- and ticagrelor-treated patients with ACS. Despite treatment with aspirin and ticagrelor, platelet mechanisms that are mediated by GPVI were still active, and platelets were able to aggregate in response to collagen and formed thrombi on collagen under flow conditions (Figures 5A–F). The addition of glenzocimab further reduced collagen and CRP-induced platelet aggregation by approximately 50% and 100%, respectively (Figure 5A–C). Eptifibatide fully inhibited both collagen- and CRP-induced aggregation (Figure 5A–C). The addition of glenzocimab also provided further inhibition of platelet adhesion and aggregation on collagen under flow conditions, which was completely blocked by the addition of eptifibatide (Figure 5D, E). Furthermore, peak thrombin generation in response to tissue factor was reduced by approximately 50% by both glenzocimab and eptifibatide (Figure 5G, H).

3.6 | Despite comparable antithrombotic effects, glenzocimab has markedly less effect on general hemostasis assessed by ROTEM compared with a GPIIb/IIIa inhibitor

ROTEM assesses clot viscoelastic properties under low shear conditions and is an indicator of general hemostasis because it is mediated by the coagulation cascade as well as platelets. ROTEM is therefore recommended for monitoring hemostasis during cardiac surgery by the National Institute for Health and Care Excellence, UK [27]. A clot formation time (CFT) of >159 seconds and maximum clot firmness (MCF) <50 mm are both associated with an approximately 4-fold increase in risk of major bleeding [27]. Although ROTEM is one of the only available clinically recommended assessments of hemostasis, it is relatively insensitive, and it has previously been shown that there is only a minimal role of TxA_2 and adenosine diphosphate-dependent mechanisms and patients with clinically relevant plasma levels of nonvitamin K oral anticoagulants may have normal values [28,29].

Glenzocimab, aspirin, and ticagrelor had no effect on CFT as assessed by the intrinsic pathway (INTEM) or extrinsic pathway (EXTEM) in blood from healthy donors (Figure 6A, B). Conversely, eptifibatide dramatically prolonged CFT to a mean of >300 seconds, as assessed by both INTEM and EXTEM (Figure 6A, B). Similarly, none of glenzocimab, aspirin, or ticagrelor affected MCF assessed by EXTEM and INTEM (Figure 6C, D). In contrast, eptifibatide reduced MCF when added to blood from healthy donors to a mean of <50 mm (Figure 6C, D). These findings were confirmed by adding glenzocimab or eptifibatide *ex vivo* to the blood of patients treated with aspirin and ticagrelor. Glenzocimab did not affect CFT or MCF as assessed by

atherosclerotic plaque under arterial shear (1000 s^{-1}) with less effect of eptifibatide 9 μM combined with aspirin and ticagrelor. (C) Representative images for platelet-thrombus formation and adhesion on a plaque under flow. Scale bar: 50 μm. (D) Representative images for P-selectin expression on a plaque under flow at 0, 3, and 10 minutes (indicated above the figure). Scale bar: 50 μm. (E) Glenzocimab 50 μg/mL marginally reduces washed human platelet spreading on 70 μg/mL atherosclerotic plaque as assessed by the total surface area of spread platelets, whereas eptifibatide 9 μM and dasatinib 10 μM significantly reduced platelet spreading. (F) Combining glenzocimab with either the combination of ASA 30 μM and ticagrelor 1 μM or 6F1 10 μg/mL significantly reduces static adhesion of platelets on 70 μg/mL atherosclerotic plaque as reflected by the number of adherent platelets. However, eptifibatide 9 μM alone shows only marginal inhibition in static conditions, and it required a combination with ASA and ticagrelor to significantly inhibit static platelet adhesion. Dasatinib 10 μM completely blocked platelet adhesion. (G) Representative images for platelet spreading and adhesion under static conditions. Scale bar: 50 μm. The results are presented as mean ± SEM (n = 6 for platelet-thrombus formation and adhesion under flow, n = 4 for P-selectin expression under flow, and n = 4 for platelet spreading). The effect of treatment groups was compared with the uninhibited sample (control) using 1- or 2-way ANOVA followed by Dunnett's correction for multiple comparisons (*P < .05 and **P < .01).

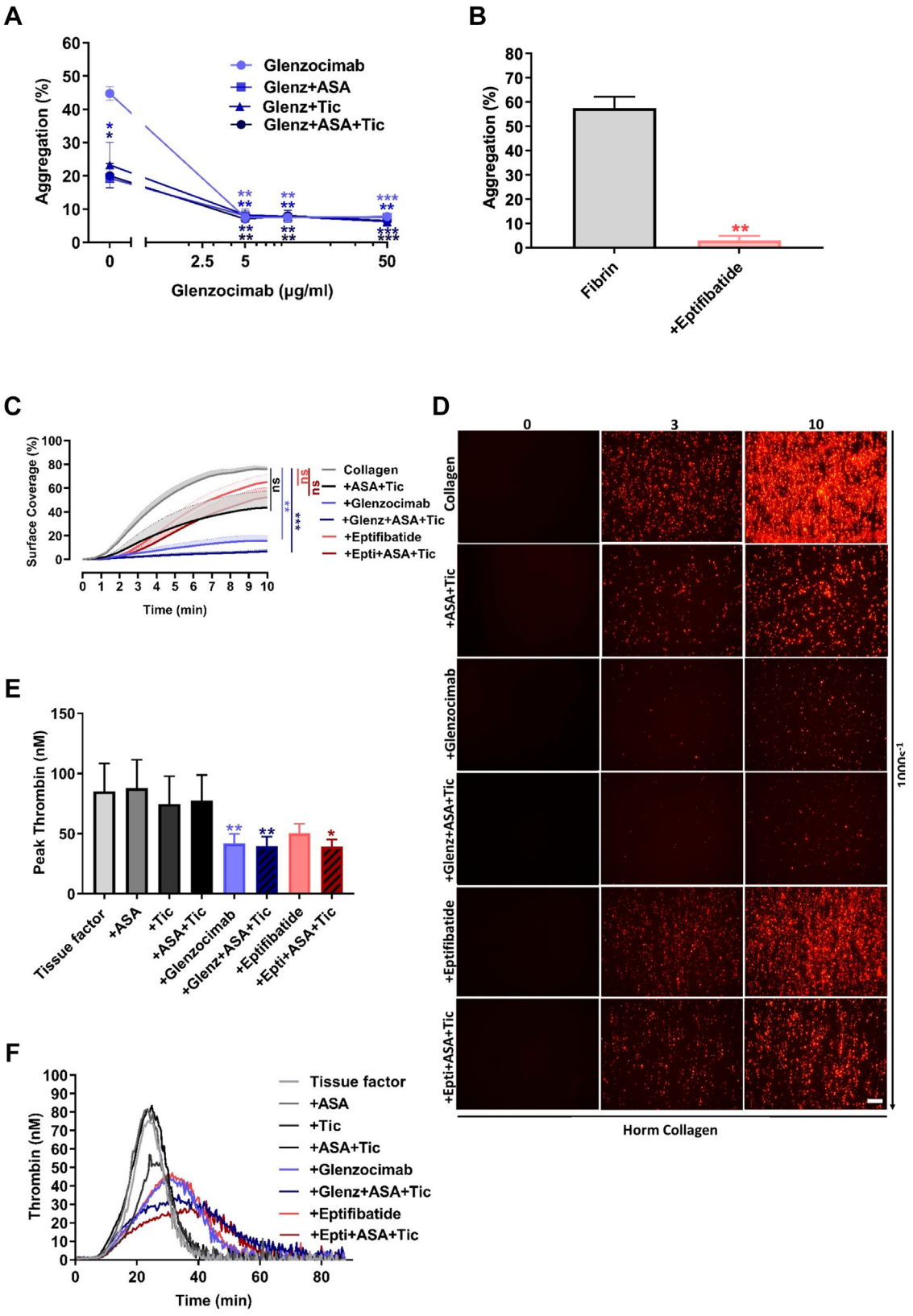

FIGURE 4 Glenzocimab blocks fibrin-mediated platelet aggregation and platelet procoagulant responses. (A) Glenzocimab at 5 µg/mL inhibited fibrin-stimulated platelet aggregation more than the combined use of ASA 30 µM and ticagrelor 1 µM as assessed in PRP by light transmission aggregometry (LTA). (B) Eptifibatide 9 µM blocked fibrin-induced platelet aggregation in PRP assessed by LTA. (C) Glenzocimab 50 µg/mL significantly blocks phosphatidyl serine (PS) exposure in flowing blood over 200 µg/mL collagen at 1000 s^{-1} as measured by Annexin V, whereas ASA 30 µM, ticagrelor 1 µM, and eptifibatide 9 µM alone did not inhibit PS exposure. The addition of ASA and ticagrelor on top of

INTEM or EXTEM, whereas eptifibatide again had a large effect that was similar to the aforementioned results in healthy volunteers (Figure 6E–H).

3.7 | GPVI depletion prevents microvascular thrombosis in a murine model of myocardial infarction

We used an intravital model of ischemia-reperfusion injury that models STEMI in a transgenic GPVI-deficient mouse [30]. The left anterior descending coronary artery was ligated for 45 minutes, and following the release of the ligature, microvascular thrombosis was observed in WT mice by the accumulation of platelets (Figure 7A, B). Microvascular thrombosis was significantly lower in GPVI-deficient mice than in WT mice (Figure 7A, D). At 30 to 60 minutes post-reperfusion, platelet accumulation was over 4-fold lower in GPVI-deficient mice ($P < .01$; Figure 7A, B, D). There was no significant effect of GPVI deficiency on neutrophil accumulation ($P = .08$; Figure 7B–D).

4 | DISCUSSION

Microvascular obstruction occurs in up to 40% of patients with STEMI treated with primary percutaneous coronary intervention despite dual antiplatelet therapy consisting of aspirin and a potent $P2Y_{12}$ inhibitor [1]. The addition of platelet GPIIb/IIIa inhibitors may help to reduce microvascular obstruction mediated by microvascular thrombosis, but they are not recommended for routine use due to their significant effect on general hemostasis [31]. We therefore investigated whether a novel platelet GPVI inhibitor, glenzocimab, provides additional antithrombotic effects when used with aspirin and a potent $P2Y_{12}$ inhibitor but with less effect on general hemostasis than a GPIIb/IIIa inhibitor.

The main findings of this study are as follows: (a) aspirin and ticagrelor only achieve partial inhibition of atherosclerotic plaque-induced platelet responses; (b) atherosclerotic plaque-induced platelet responses are critically dependent on platelet GPVI and are potently inhibited by a novel GPVI inhibitor, glenzocimab; (c) glenzocimab provides multiple additional antithrombotic effects (inhibition of platelet aggregation, platelet adhesion, and thrombin generation) when added to the blood of aspirin and ticagrelor-treated patients with ACS *ex vivo*; (d) glenzocimab has less effect on general hemostasis than the GPIIb/IIIa inhibitor eptifibatide; and (e) depletion of GPVI in an animal model of myocardial infarction reduces microvascular thrombosis.

Aspirin and potent $P2Y_{12}$ inhibitors have greatly improved patient outcomes following ACS by inhibiting platelet pathways that are mediated by TxA_2 and adenosine diphosphate, respectively. These secondary mediators have a major role in amplifying platelet activation through G protein-coupled receptors (GPCRs). Inhibition of these central pathways provides partial inhibition of a wide range of different platelet pathways [32], leading to broad antithrombotic effects and widespread effects on hemostasis. However, in patients with STEMI, treatment with aspirin, a potent $P2Y_{12}$ inhibitor, and a parenteral anticoagulant is not always sufficient for the treatment of microvascular thrombosis and thromboinflammation caused by embolization of thrombus and atherosclerotic plaque material. Indeed, a recent study demonstrated that achieving high levels of $P2Y_{12}$ inhibition during STEMI with the potent $P2Y_{12}$ inhibitor cangrelor did not reduce microvascular obstruction [33]. Our results demonstrate that aspirin and ticagrelor only provide partial inhibition of atherosclerotic plaque-mediated platelet responses, thereby providing an explanation for their observed lack of efficacy in preventing microvascular obstruction in STEMI. We demonstrated that atherosclerotic plaque-mediated platelet responses were mediated almost exclusively by GPVI, which is an immunoreceptor tyrosine-based activation motif (ITAM)-coupled receptor, rather than a GPCR. This is in agreement with several previous studies that have demonstrated this critical role of GPVI in atherosclerotic plaque-induced platelet responses with additional but less critical roles of GPIb, α2β1, and $P2Y_{12}$ [6,7,19–21,34]. Although aspirin and $P2Y_{12}$ inhibitors potently downregulate the response to a number of different GPCRs, they have less impact on activation of ITAM-coupled receptors, in keeping with previous studies [19,35,36]. We noted that $P2Y_{12}$ inhibition still had a moderate effect on atherosclerotic plaque-induced adhesion and thrombus formation under flow conditions, however, in keeping with a prior study that used the potent $P2Y_{12}$ inhibitor ARC69931MX (cangrelor) [20]. In contrast to this study, we saw less overall effect of ticagrelor on atherosclerotic plaque-induced platelet responses, particularly in aggregation assays, which is likely to be due to differences in plaque preparation or could be related to less complete $P2Y_{12}$ inhibition or differences in mechanisms of action of ticagrelor compared to cangrelor [37]. Recent studies, including our own, have also shown that Syk and Btk inhibitors can inhibit downstream signaling of GPVI, thereby inhibiting atherosclerotic plaque-induced platelet responses [34,38–41]. Glenzocimab appears more potent than clinically available Btk and Syk inhibitors investigated in these *in vitro* studies; however, the effects of Btk inhibitors may compound

glenzocimab amplified its effect on PS exposure. (D) Representative images for Annexin V measurement in flowing hirudin-anticoagulated blood over collagen at 0, 3, and 10 minutes (indicated above the figure). Scale bar: 50 μm. (E) Glenzocimab significantly reduces peak thrombin-induced by 1 pM tissue factor as assessed by calibrated automated thrombogram (CAT, Stago), whereas there was no effect of ASA 30 μM and ticagrelor 1 μM. Eptifibatide 9 μM significantly reduced peak thrombin when combined with ASA and ticagrelor. (F) A representative thrombogram for thrombin generation was assessed by CAT. The results are shown as mean ± SEM ($n = 4$ for polymerized fibrin-stimulated platelet aggregation, $n = 4$ for Annexin V measurement under flow, and $n = 7$ for thrombin generation). The effect of the treatment group was compared with the uninhibited samples (vehicle control) using 1- or 2-way ANOVA followed by Dunnett's correction for multiple comparisons (*$P < .05$, **$P < .01$, and ***$P < .001$). PRP, platelet-rich plasma.

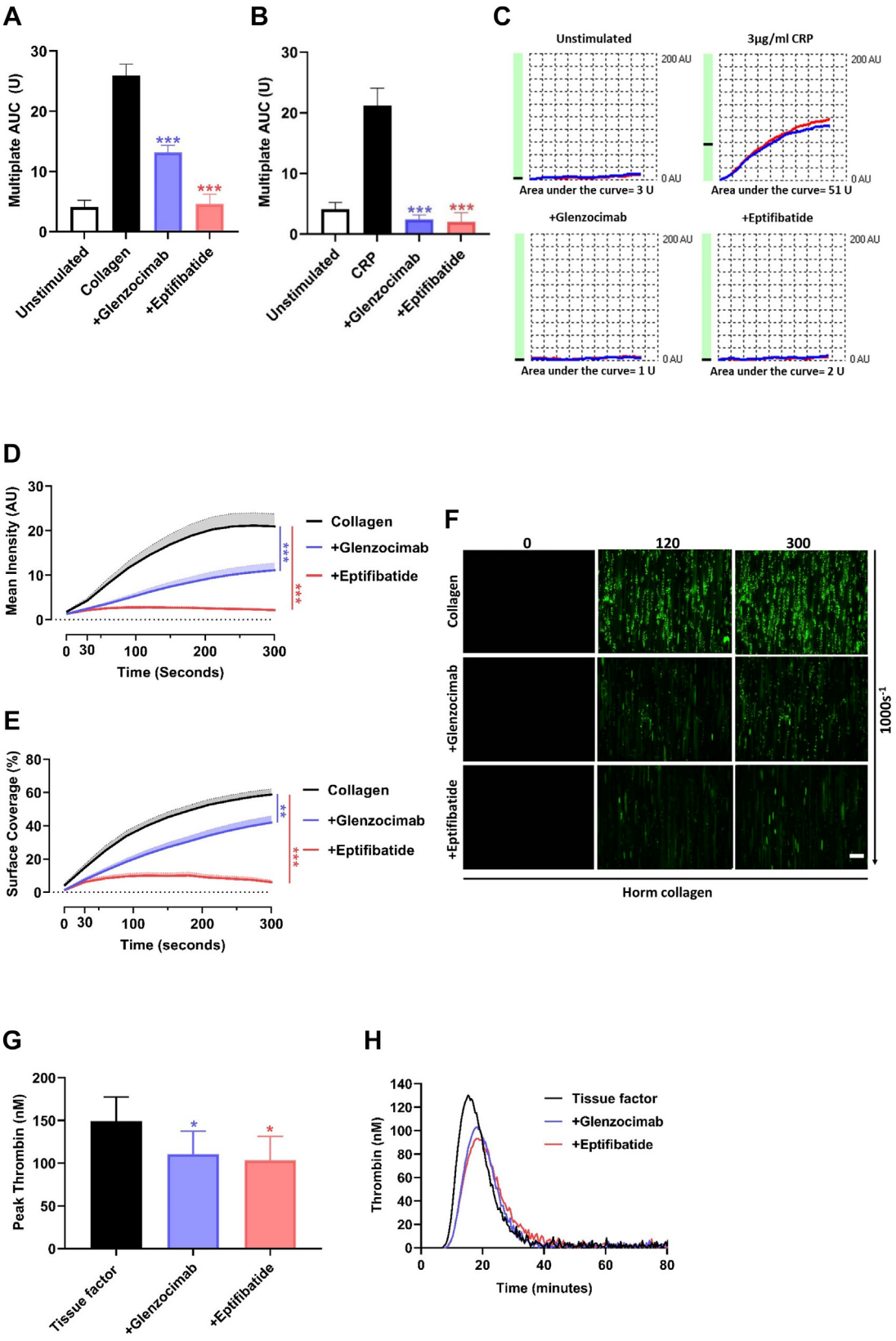

FIGURE 5 Amplified effects of glenzocimab on *ex vivo* platelet responses in aspirin- and ticagrelor-treated patients. (A) Glenzocimab 50 µg/mL provided additional inhibition of 3.2 µg/mL Horm collagen-induced platelet aggregation in patients treated with aspirin and ticagrelor. Eptifibatide 9 µM completely blocked collagen-stimulated platelet aggregation *ex vivo*. (B) Glenzocimab 50 µg/mL completely blocked 3 µg/mL collagen-related peptide (CRP)-stimulated platelet aggregation *ex vivo* in samples from aspirin- and ticagrelor-treated patients. (C) Representative aggregation traces for CRP-induced platelet aggregation. (D) Glenzocimab 50 µg/mL significantly decreased platelet-thrombus

over time when given clinically as they have an irreversible effect on platelets, thereby preventing direct comparisons of potency.

The specific GPVI inhibitor glenzocimab provided potent inhibition of atherosclerotic plaque-induced platelet responses and provided multiple additional antithrombotic effects when added to the blood of aspirin- and ticagrelor-treated patients with ACS. We also demonstrated that GPVI inhibition by glenzocimab provides mild anticoagulant effects in the form of reduced thrombin generation mediated by a reduction in fibrin-mediated platelet activation. Furthermore, we demonstrated the *in vivo* relevance of these mechanisms by showing that GPVI plays a critical role in microvascular thrombosis in an animal model of STEMI and ischemia-reperfusion. Taken together, these findings suggest that GPVI inhibition could potentially reduce 2 separate but equally important pathophysiological mechanisms during STEMI: atherosclerotic plaque-induced platelet activation and microvascular thrombosis triggered by ischemia-reperfusion injury.

The effects of glenzocimab shared many similarities with eptifibatide, which inhibits the platelet GPIIb/IIIa receptor (also known as αIIbβ3). The main antiplatelet effects of GPIIb/IIIa inhibitors are traditionally attributed to blockade of GPIIb/IIIa inside-out signaling, preventing cross-linking of GPIIb/IIIa by fibrinogen, thereby preventing platelet aggregation. However, GPIIb/IIIa also acts in conjunction with GPVI to signal via ITAM when activated by fibrinogen and mediates a wide range of distinct mechanisms, including platelet spreading and stable thrombus formation [30,42–44]. Indeed, glenzocimab and eptifibatide both provided similar inhibition of atherosclerotic plaque-induced platelet aggregation and adhesion, as well as fibrin-induced platelet aggregation and thrombin generation. It has previously been shown that eptifibatide can promote disaggregation of formed platelet aggregates [45]. We demonstrated that both glenzocimab and eptifibatide could cause disaggregation of platelets that had already been triggered by atherosclerotic plaque material. This is consistent with another recent report on the ability of glenzocimab to promote platelet disaggregation [17] and demonstrates potential benefits of a GPVI inhibitor following atherosclerotic plaque rupture in STEMI. Of note, eptifibatide provided greater inhibition of Horm collagen-induced platelet responses than glenzocimab. This is explained by our results showing that platelet responses to Horm collagen are not mediated purely by GPVI but also have an important contribution from α2β1 (a recent report also demonstrated a role of GPR56 [46]). In contrast, atherosclerotic plaque-mediated responses were overall mostly mediated by GPVI. Our experiments were performed using homogenized pooled plaque material from multiple donors and therefore demonstrated the overall mechanisms involved in atherosclerotic plaque-induced platelet responses. However, plaque heterogeneity, exposure of collagen, and lesion rupture may all influence the differential role of mechanisms of platelet activation and are worthy of further investigation.

In contrast to their similar antithrombotic effects, eptifibatide had a dramatic effect on general hemostasis assessed by ROTEM, while glenzocimab had no measurable effect. Although this demonstrates the marked effect of GPIIb/IIIa inhibitors on hemostasis, it does not rule out an effect of glenzocimab, as aspirin and ticagrelor also did not affect the ROTEM measurements despite their known effect on hemostasis. However, these findings were also in keeping with the well-established profound hemostatic defect caused by inhibition or deficiency of GPIIb/IIIa and the minor effect of GPVI deficiency on hemostasis [11–14]. GPIIb/IIIa inhibitors, $P2Y_{12}$ inhibitors, and aspirin have all achieved potent platelet inhibition by combining broad effects on multiple platelet activation pathways that are involved in both thrombosis and hemostasis. Instead, GPVI inhibition offers a more selective approach of inhibiting a single pathway with greater potency to specifically reduce atherosclerotic plaque- and fibrin-induced platelet responses and leave general hemostasis intact. The minor role of GPVI in general hemostasis is supported by previous studies that have shown no effect of glenzocimab on hemostasis in nonhuman primates and in a phase 1 clinical trial [15]. Providing additional reassurance, a patient with GPVI deficiency did not display any signs of bleeding when treated with dual antiplatelet therapy [47].

There are currently 2 GPVI inhibitors that are being tested in clinical trials: glenzocimab and revacept. Revacept is a dimeric fusion protein of the GPVI extracellular domain and the human Fc-fragment, which mimics dimeric GPVI and binds to exposed GPVI agonists, such as collagen [48–50]. Revacept indirectly blocks access of dimeric platelet GPVI to agonists but does not directly block the function of the GPVI receptor and does not bind to monomeric GPVI or block monomeric GPVI-mediated responses induced by fibrin [23]. In the recent ISAR-PLASTER study, revacept did not reduce troponin release following percutaneous coronary intervention for stable coronary artery disease [51]. Potentially explaining these results, revacept is thought to only inhibit dimeric, but not monomeric, GPVI-mediated platelet responses and offers substantially less potent inhibition than antibody-based approaches [7,23]. Our current results demonstrate that atherosclerotic plaque activates both the monomeric and

growth (assessed by fluorescence intensity) on 200 μg/mL Horm collagen in flowing blood perfused at 1000 s^{-1} from patients treated with aspirin and ticagrelor *ex vivo*. (E) Glenzocimab 50 μg/mL additionally reduced platelet adhesion (assessed by platelet coverage area) on 200 μg/mL Horm collagen in flowing blood from patients treated with ASA and ticagrelor. (F) Representative images for platelet-thrombus formation and adhesion under flow. Scale bar: 50 μm. (G) Glenzocimab 50 μg/mL and eptifibatide 9 μM significantly reduced peak thrombin generation initiated by 1 pM tissue factor in platelet-rich plasma (PRP) samples from patients with acute coronary syndrome treated with aspirin and ticagrelor. (H) Representative thrombogram. The results are presented as mean ± SEM (n = 21 for multiple electrode aggregometry aggregation, n = 18 for flow adhesion, and n = 7 for thrombin generation). The effect of spiking samples with glenzocimab (50 μg/mL) and eptifibatide (9 M) was compared with the unspiked sample (vehicle control) using a linear mixed model with Dunnett's correction for multiple comparisons (*$P < .05$, **$P < .01$, and ***$P < .001$). AUC, area under the curve.

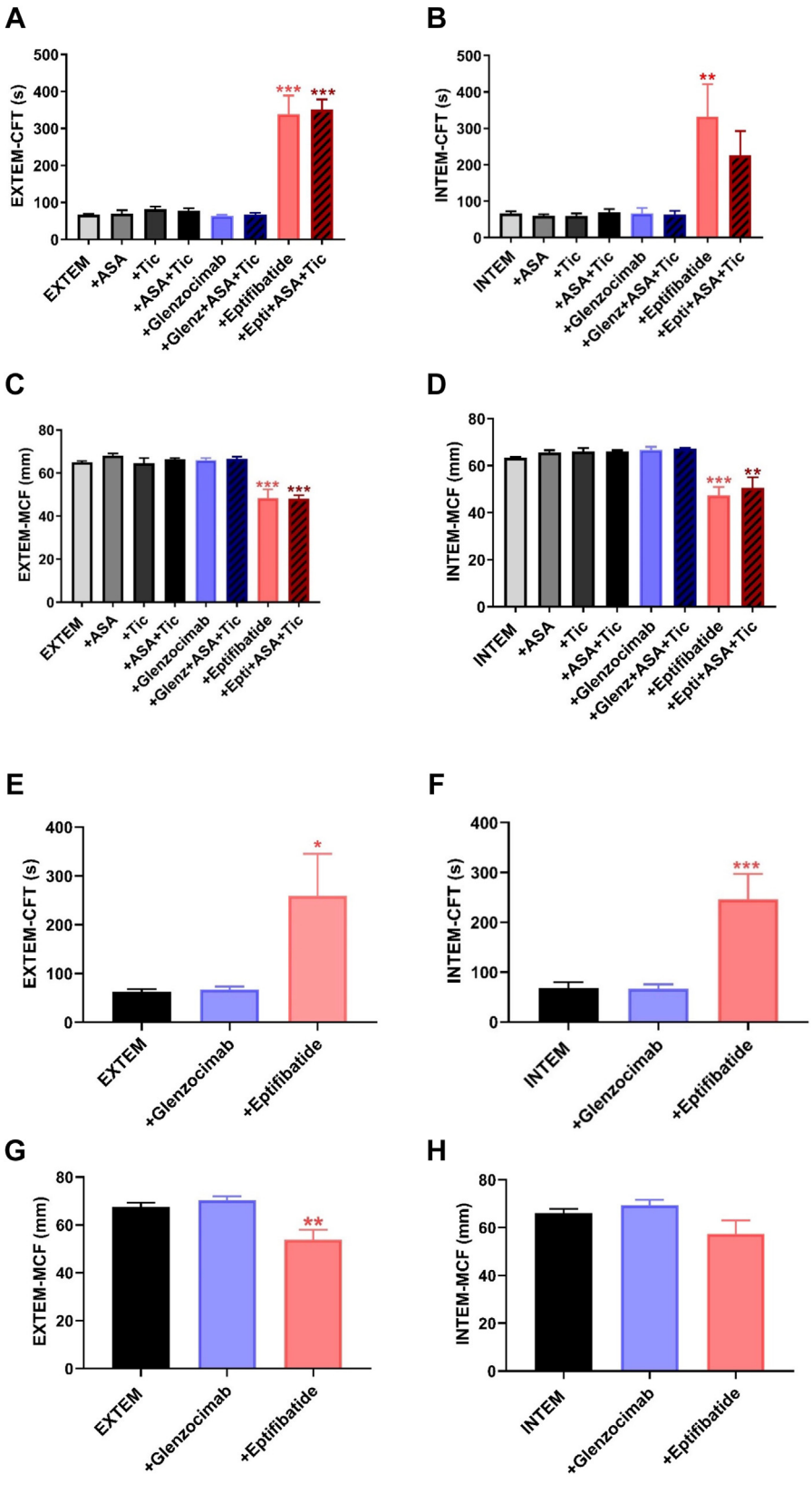

FIGURE 6 Glenzocimab has less effect on hemostasis (assessed by rotational thromboelastometry) than eptifibatide. (A) There was no effect of glenzocimab 50 μg/mL (even when combined with aspirin 30 μM and ticagrelor 1 μM) on clot formation time (CFT) on whole blood coagulation stimulated via the extrinsic pathway (EXTEM). In contrast, eptifibatide (9 μM) dramatically increased the EXTEM-CFT as assessed by rotational thromboelastometry in healthy donors *in vitro*. (B) Similarly, glenzocimab, aspirin, and ticagrelor had no effect on CFT stimulated via the intrinsic pathway (INTEM), whereas eptifibatide dramatically increased the INTEM-CFT in healthy donors *in vitro*. (C) Glenzocimab, aspirin, and ticagrelor did not affect maximum clot firmness (MCF) in whole blood coagulation stimulated via EXTEM. In contrast, eptifibatide significantly decreased EXTEM-MCF in healthy donors *in vitro*. (D) Glenzocimab, aspirin, and ticagrelor also did not affect MCF stimulated via INTEM. Again, eptifibatide significantly decreased INTEM-MCF in healthy donors *in vitro*. (E) Glenzocimab 50 μg/mL did not affect EXTEM-CFT, whereas eptifibatide 9 μM significantly increased EXTEM-CFT *ex vivo* in whole blood from patients with acute coronary syndrome (ACS) treated with aspirin and ticagrelor. (F) Similarly, glenzocimab 50 μg/mL did not affect INTEM-CFT, whereas eptifibatide 9 μM significantly increased INTEM-CFT *ex vivo* in whole blood from patients with ACS treated with aspirin and ticagrelor. (G) Glenzocimab 50 μg/mL did not affect EXTEM-MCF, which was significantly decreased by eptifibatide 9 μM *ex vivo* in whole blood from patients with ACS treated with aspirin and ticagrelor. (H) Both glenzocimab 50 μg/mL and eptifibatide 9 μM did not significantly affect INTEM-MCF *ex vivo* in whole blood from patients with ACS treated with aspirin and ticagrelor. The results are presented as mean ± SEM (n = 3 for healthy donors and n = 8 for patients with ACS). The effect of different treatments was compared with the unspiked samples by 1-way ANOVA followed by Dunnett's correction for multiple comparisons (*P < .05, **P < .01, and ***P < .001).

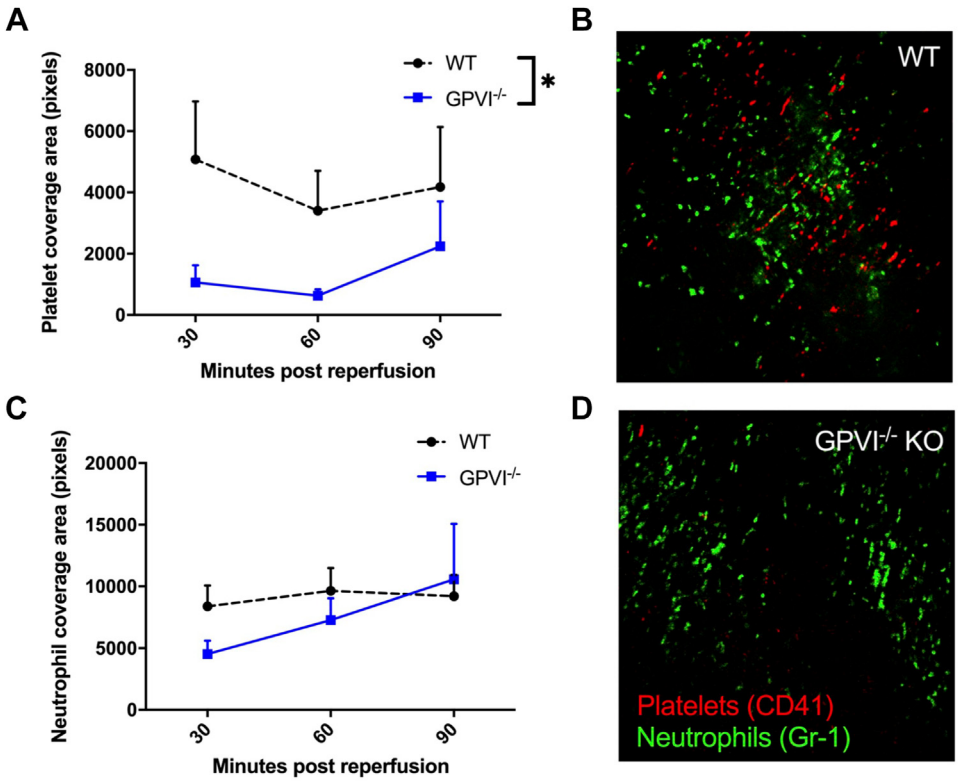

FIGURE 7 Genetic depletion of glycoprotein (GP) VI reduced microvascular thrombosis in a murine model of ST-elevation myocardial infarction and ischemia-reperfusion injury. A time course of intravital imaging of the beating heart was obtained using an upright intravital microscope focused on a stabilized region of the beating left ventricle and its microvasculature. (A) After 45 minutes of ligation of the left anterior descending coronary artery, the ligature was removed, and the heart was reperfused, with significantly reduced platelet deposition in the coronary microvessels of GPVI-depleted mice compared with wild-type (WT) mice. (B) Representative image of microvascular deposition of platelets (red) and neutrophils (green) in WT mice. (C) There was no significant effect of the genetic depletion of GPVI on neutrophil deposition. (D) Representative image of microvascular deposition of platelets (red) and neutrophils (green) in mice with GPVI genetic depletion. WT (n = 10) compared with GPVI-deficient mice (n = 6) using 2-way ANOVA (*P < .05, **P < .01, and ***P < .001).

dimeric forms of GPVI, and we have also previously shown that inhibition of monomeric GPVI is required for inhibition of fibrin-mediated platelet responses [23]. For a GPVI inhibitor to fully inhibit atherosclerotic plaque- and fibrin-induced platelet responses, inhibition of both monomeric and dimeric GPVI is likely to be necessary, as achieved by glenzocimab in this study.

In summary, the addition of a novel GPVI inhibitor, glenzocimab, to aspirin and ticagrelor provides amplified inhibition of multiple critical mechanisms of atherothrombosis, including platelet activation in response to atherosclerotic plaque. Glenzocimab and the GPIIb/IIIa inhibitor, eptifibatide, share many similar antithrombotic effects, although glenzocimab has less impact on mechanisms involved in hemostasis than eptifibatide.

AUTHOR CONTRIBUTIONS

F.A. contributed to the design of the study, performed experiments, analyzed results, interpreted data, and drafted and reviewed/revised the manuscript. M.H., J.P., and A.S. performed experiments and reviewed/revised the manuscript. D.K. performed experiments, analyzed results, and reviewed/revised the manuscript. P.B., O.H., A.T., and D.C. recruited patients and reviewed/revised the manuscript. P.H., P.N., P.K., N.K., M.J.P., P.M., and S.P.W. contributed to the design of the study and reviewed/revised the manuscript. M.T. conceptualized and designed the study and reviewed/revised the manuscript.

DECLARATION OF COMPETING INTERESTS

P.L.R.N. reports institutional research grants from Novartis, Rigel Pharmaceuticals, Principia Biopharma, Acticor Biotech, and honoraria from Bayer, Grifols, and Takeda. P.K. receives research support for basic, translational, and clinical research projects from the European Union, British Heart Foundation, Leducq Foundation, Medical Research Council (UK), German Centre for Cardiovascular Research, and several drug and device companies active in atrial fibrillation, and has received honoraria from several such companies in the past, but not in the last 3 years. P.K. is listed as an inventor on 2 patents held by the University of Birmingham (Atrial Fibrillation Therapy WO 2015140571 and Markers for Atrial Fibrillation WO 2016012783). M.J.-P. is the founder of Acticor Biotech and scientific adviser of the company. S.P.W. reports institutional research grants from Acticor Biotech, Novartis, Rigel Pharmaceuticals, and Principia Biopharma.

M.R.T. declares institutional research grants from Acticor Biotech, Novartis, Rigel Pharmaceuticals, and Principia Biopharma. M.R.T. declares consultancy fees from Cytosorbents. F.O.A., M.H.H., D.P.K., J.P., P.B., O.H., P.H., A.S., N.K., A.T., and P.H.M. have no competing interests to disclose.

REFERENCES

[1] Eitel I, Waha S de, Wöhrle J, Fuernau G, Lurz P, Pauschinger M, Desch S, Schuler G, Thiele H. Comprehensive prognosis assessment by CMR imaging after ST-segment elevation myocardial infarction. *J Am Coll Cardiol*. 2014;64:1217–26.

[2] Niccoli G, Scalone G, Lerman A, Crea F. Coronary microvascular obstruction in acute myocardial infarction. *Eur Heart J*. 2016;37:1024–33.

[3] Padro T, Manfrini O, Bugiardini R, Canty J, Cenko E, Luca GD, Duncker DJ, Eringa EC, Koller A, Tousoulis D, Trifunovic D, Vavlukis M, Wit C de, Badimon L. ESC Working Group on Coronary Pathophysiology and Microcirculation position paper on 'coronary microvascular dysfunction in cardiovascular disease.' *Cardiovasc Res*. 2020;116:741–55.

[4] Thiele H, Schindler K, Friedenberger J, Eitel I, Furnau G, Grebe E, Erbs S, Linke A, Mobius-Winkler S, Kivelitz D, Schuler G. Intracoronary compared with intravenous bolus abciximab application in patients with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention: the randomized Leipzig immediate percutaneous coronary intervention abciximab IV versus IC in ST-elevation myocardial infarction trial. *Circulation*. 2008;118:49–57.

[5] Tavenier AH, Hermanides RS, Fabris E, Lapostolle F, Silvain J, Berg ten JM, Lassen JF, Bolognese L, Cantor WJ, Cequier Á, Chettibi M, Goodman SG, Hammett CJ, Huber K, Janzon M, Merkely B, Storey RF, Zeymer U, Ecollan P, Collet JP, et al. Efficacy and safety of glycoprotein IIb/IIIa inhibitors on top of ticagrelor in STEMI: a subanalysis of the ATLANTIC trial. *Thromb Haemost*. 2020;120:65–74.

[6] Alenazy FO, Thomas MR. Novel antiplatelet targets in the treatment of acute coronary syndromes. *Platelets*. 2021;32:15–28.

[7] Jamasbi J, Megens RTA, Bianchini M, Münch G, Ungerer M, Faussner A, Sherman S, Walker A, Goyal P, Jung S, Brandl R, Weber C, Lorenz R, Farndale R, Elia N, Siess W. Differential inhibition of human atherosclerotic plaque-induced platelet activation by dimeric GPVI-Fc and anti-GPVI antibodies: functional and imaging studies. *J Am Coll Cardiol*. 2015;65:2404–15.

[8] Alshehri OM, Hughes CE, Montague S, Watson SK, Frampton J, Bender M, Watson SP. Fibrin activates GPVI in human and mouse platelets. *Blood*. 2015;126:1601–8.

[9] Mammadova-Bach E, Ollivier V, Loyau S, Schaff M, Dumont B, Favier R, Freyburger G, Latger-Cannard V, Nieswandt B, Gachet C, Mangin PH, Jandrot-Perrus M. Platelet glycoprotein VI binds to polymerized fibrin and promotes thrombin generation. *Blood*. 2015;126:683–91.

[10] Pachel C, Mathes D, Arias-Loza AP, Heitzmann W, Nordbeck P, Deppermann C, Lorenz V, Hofmann U, Nieswandt B, Frantz S. Inhibition of platelet GPVI protects against myocardial ischemia-reperfusion injury. *Arterioscler Thromb Vasc Biol*. 2016;36:629–35.

[11] Lockyer S, Okuyama K, Begum S, Le S, Sun B, Watanabe T, Matsumoto Y, Yoshitake M, Kambayashi J, Tandon NN. GPVI-deficient mice lack collagen responses and are protected against experimentally induced pulmonary thromboembolism. *Thromb Res*. 2006;118:371–80.

[12] Bynagari-Settipalli YS, Cornelissen I, Palmer D, Duong D, Concengco C, Ware J, Coughlin SR. Redundancy and interaction of thrombin- and collagen-mediated platelet activation in tail bleeding and carotid thrombosis in mice. *Arterioscler Thromb Vasc Biol*. 2014;34:2563–9.

[13] Jandrot-Perrus M, Hermans C, Mezzano D. Platelet glycoprotein VI genetic quantitative and qualitative defects. *Platelets*. 2019;30:708–13.

[14] Nurden AT. Glanzmann thrombasthenia. *Orphanet J Rare Dis*. 2006;1:10.

[15] Voors-Pette C, Lebozec K, Dogterom P, Jullien L, Billiald P, Ferlan P, Renaud L, Favre-Bulle O, Avenard G, Machacek M, Pletan Y, Jandrot-Perrus M. Safety and tolerability, pharmacokinetics, and pharmacodynamics of ACT017, an antiplatelet GPVI (glycoprotein VI) Fab. *Arterioscler Thromb Vasc Biol*. 2019;39:956–64.

[16] Lebozec K, Jandrot-Perrus M, Avenard G, Favre-Bulle O, Billiald P. Quality and cost assessment of a recombinant antibody fragment produced from mammalian, yeast and prokaryotic host cells: a case study prior to pharmaceutical development. *N Biotechnol*. 2018;44:31–40.

[17] Ahmed MU, Kaneva V, Loyau S, Nechipurenko D, Receveur N, Le Bris ML, Janus-Bell E, Didelot M, Rauch A, Susen S, Chakfé N, Lanza F, Gardiner EE, Andrews RK, Panteleev M, Gachet C, Jandrot-Perrus M, Mangin PH. Pharmacological blockade of glycoprotein VI promotes thrombus disaggregation in the absence of thrombin. *Arterioscler Thromb Vasc Biol*. 2020;40:2127–42.

[18] Mazighi M, Peeters A, Richard S, Molina C, Lemmens R, Toni D, Plétan Y, Jandrot-Perrus M, Comenducci A, Avenard G, Lyrer P, Kohrmann M, Group AS. ACTIMIS trial: safety interim analysis data of Glenzocimab, a novel antiplatelet agent on top of acute ischemic stroke standard of care. *ISTH 2021 Congress*. 2021.

[19] Penz SM, Reininger AJ, Toth O, Deckmyn H, Brandl R, Siess W. Glycoprotein Ibalpha inhibition and ADP receptor antagonists, but not aspirin, reduce platelet thrombus formation in flowing blood exposed to atherosclerotic plaques. *Thromb Haemost*. 2007;97:435–43.

[20] Reininger AJ, Bernlochner I, Penz SM, Ravanat C, Smethurst P, Farndale RW, Gachet C, Brandl R, Siess W. A 2-step mechanism of arterial thrombus formation induced by human atherosclerotic plaques. *J Am Coll Cardiol*. 2010;55:1147–58.

[21] Cosemans JMEM, Kuijpers MJE, Lecut C, Loubele STBG, Heeneman S, Jandrot-Perrus M, Heemskerk JWM. Contribution of platelet glycoprotein VI to the thrombogenic effect of collagens in fibrous atherosclerotic lesions. *Atherosclerosis*. 2005;181:19–27.

[22] Kavanagh DPJ, Lokman AB, Neag G, Colley A, Kalia N. Imaging the injured beating heart intravitally and the vasculoprotection afforded by haematopoietic stem cells. *Cardiovasc Res*. 2019;115:1918–32.

[23] Onselaer MB, Hardy AT, Wilson C, Sanchez X, Babar AK, Miller JLC, Watson CN, Watson SK, Bonna A, Philippou H, Herr AB, Mezzano D, Ariens RAS, Watson SP. Fibrin and D-dimer bind to monomeric GPVI. *Blood Adv*. 2017;1:1495–504.

[24] Nagelschmitz J, Blunck M, Kraetzschmar J, Ludwig M, Wensing G, Hohlfeld T. Pharmacokinetics and pharmacodynamics of acetylsalicylic acid after intravenous and oral administration to healthy volunteers. *Clin Pharmacol*. 2014;6:51–9.

[25] Dobesh PP, Oestreich JH. Ticagrelor: pharmacokinetics, pharmacodynamics, clinical efficacy, and safety. *Pharmacotherapy*. 2014;34:1077–90.

[26] Lecut C, Feeney LA, Kingsbury G, Hopkins J, Lanza F, Gachet C, Villeval JL, Jandrot-Perrus M. Human platelet glycoprotein VI function is antagonized by monoclonal antibody-derived Fab fragments. *J Thromb Haemost*. 2003;1:2653–62.

[27] Whiting P, Al M, Westwood M, Ramos IC, Ryder S, Armstrong N, Misso K, Ross J, Severens J, Kleijnen J. Viscoelastic point-of-care testing to assist with the diagnosis, management and monitoring of haemostasis: a systematic review and cost-effectiveness analysis. *Health Technol Asses*. 2015;19:1–228.

[28] Scharbert G, Auer A, Kozek-Langenecker S. Evaluation of the Platelet Mapping Assay on rotational thromboelastometry ROTEM. *Platelets*. 2009;20:125–30.

[29] Sahli SD, Castellucci C, Roche TR, Rössler J, Spahn DR, Kaserer A. The impact of direct oral anticoagulants on viscoelastic testing – a systematic review. *Front Cardiovasc Med*. 2022;9:991675.
[30] Mangin PH, Onselaer MB, Receveur N, Le Lay NL, Hardy AT, Wilson C, Sanchez X, Loyau S, Dupuis A, Babar AK, Miller JL, Philippou H, Hughes CE, Herr AB, Ariëns RA, Mezzano D, Jandrot-Perrus M, Gachet C, Watson SP. Immobilized fibrinogen activates human platelets through glycoprotein VI. *Haematologica*. 2018;103:898–907.
[31] Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, Caforio ALP, Crea F, Goudevenos JA, Halvorsen S, Hindricks G, Kastrati A, Lenzen MJ, Prescott E, Roffi M, Valgimigli M, Varenhorst C, Vranckx P, Widimsky P, ESC Scientific Document Group. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the task force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J*. 2018;39:119–77.
[32] Storey RF, Sanderson HM, White AE, May JA, Cameron KE, Heptinstall S. The central role of the P2T receptor in amplification of human platelet activation, aggregation, secretion and procoagulant activity. *Br J Haematol*. 2000;110:925–34.
[33] Ubaid S, Ford TJ, Berry C, Murray HM, Wrigley B, Khan N, Thomas MR, Armesilla AL, Townend JN, Khogali SS, Munir S, Martins J, Hothi SS, McAlindon EJ, Cotton JM. Cangrelor versus ticagrelor in patients treated with primary percutaneous coronary intervention: impact on platelet activity, myocardial microvascular function and infarct size: a randomized controlled trial. *Thromb Haemost*. 2019;119:1171–81.
[34] Harbi MH, Smith CW, Nicolson PLR, Watson SP, Thomas MR. Novel antiplatelet strategies targeting GPVI, CLEC-2 and tyrosine kinases. *Platelets*. 2021;32:29–41.
[35] Jamasbi J, Ayabe K, Goto S, Nieswandt B, Peter K, Siess W. Platelet receptors as therapeutic targets: past, present and future. *Thromb Haemost*. 2017;117:1249–57.
[36] Reininger AJ, Brandl R, Penz S, Goyal P, Rabie T, Rother E, Goetz C, Engelmann B, Farndale R, Nieswandt B, Siess W. Human atheromatous plaques stimulate thrombus formation by activating platelet glycoprotein VI. *Blood*. 2004;104:2623.
[37] Khalil J, Dimofte T, Roberts T, Keith M, Amaradasa K, Hindle MS, Bancroft S, Hutchinson J, Naseem K, Johnson T, Mundell SJ. Ticagrelor inverse agonist activity at the P2Y12 receptor is non-reversible versus its endogenous agonist ADP. *Br J Pharmacol*. 2023. In press.
[38] Harbi MH, Smith CW, Alenazy FO, Nicolson PLR, Tiwari A, Watson SP, Thomas MR. Antithrombotic effects of fostamatinib in combination with conventional antiplatelet drugs. *Int J Mol Sci*. 2022;23:6982.
[39] Smith CW, Harbi MH, Garcia-Quintanilla L, Rookes K, Brown H, Poulter NS, Watson SP, Nicolson PLR, Thomas MR. The Btk inhibitor AB-95-LH34 potently inhibits atherosclerotic plaque–induced thrombus formation and platelet procoagulant activity. *J Thromb Haemost*. 2022;20:2939–52.
[40] Busygina K, Denzinger V, Bernlochner I, Weber C, Lorenz R, Siess W. Btk inhibitors as first oral atherothrombosis-selective antiplatelet drugs? *Thromb Haemost*. 2019;119:1212–21.
[41] Denzinger V, Busygina K, Jamasbi J, Pekrul I, Spannagl M, Weber C, Lorenz R, Siess W. Optimizing platelet GPVI inhibition versus haemostatic impairment by the Btk inhibitors ibrutinib, acalabrutinib, ONO/GS-4059, BGB-3111 and evobrutinib. *Thromb Haemost*. 2019;119:397–406.
[42] Durrant TN, Bosch van den MT, Hers I. Integrin αIIbβ3 outside-in signaling. *Blood*. 2017;130:1607–19.
[43] Perrella G, Huang J, Provenzale I, Swieringa F, Heubel-Moenen FCJI, Farndale RW, Roest M, Meijden van der PEJ, Thomas M, Ariëns RAS, Jandrot-Perrus M, Watson SP, Heemskerk JWM. Nonredundant roles of platelet glycoprotein VI and integrin αIIbβ3 in fibrin-mediated microthrombus formation. *Arterioscler Thromb Vasc Biol*. 2021;41:e97–111.
[44] Ahmed MU, Receveur N, Janus-Bell E, Mouriaux C, Gachet C, Jandrot-Perrus M, Hechler B, Gardiner EE, Mangin PH. Respective roles of glycoprotein VI and FcγRIIA in the regulation of αIIbβ3-mediated platelet activation to fibrinogen, thrombus buildup, and stability. *Res Pract Thromb Haemost*. 2021;5:e12551.
[45] Speich HE, Earhart AD, Hill SN, Cholera S, Kueter TJ, Smith JN, White MM, Jennings LK. Variability of platelet aggregate dispersal with glycoprotein IIb–IIIa antagonists eptifibatide and abciximab. *J Thromb Haemost*. 2009;7:983–91.
[46] Yeung J, Adili R, Stringham EN, Luo R, Vizurraga A, Rosselli-Murai LK, et al. GPR56/ADGRG1 is a platelet collagen-responsive GPCR and hemostatic sensor of shear force. *Proc Natl Acad Sci U S A*. 2020;117:28275–86.
[47] Loyau S, Faille D, Gautier P, Nurden P, Jandrot-Perrus M, Ajzenberg N. Absence of bleeding upon dual antiplatelet therapy in a patient with a immune GPVI deficiency. *Platelets*. 2020;32:1–5.
[48] Jamasbi J, Megens RTA, Bianchini M, Uhland K, Münch G, Ungerer M, Sherman S, Faussner A, Brandl R, John C, Buchner J, Weber C, Lorenz R, Elia N, Siess W. Cross-linking GPVI-Fc by anti-Fc antibodies potentiates its inhibition of atherosclerotic plaque- and collagen-induced platelet activation. *JACC Basic Transl Sci*. 2016;1:131–42.
[49] Ungerer M, Rosport K, Bültmann A, Piechatzek R, Uhland K, Schlieper P, Gawaz M, Münch G. Novel antiplatelet drug revacept (dimeric glycoprotein VI-Fc) specifically and efficiently inhibited collagen-induced platelet aggregation without affecting general hemostasis in humans. *Circulation*. 2011;123:1891–9.
[50] Mojica Muñoz AK, Jamasbi J, Uhland K, Degen H, Münch G, Ungerer M, Brandl R, Megens R, Weber C, Lorenz R, Siess W. Recombinant GPVI-Fc added to single or dual antiplatelet therapy in vitro prevents plaque-induced platelet thrombus formation. *Thromb Haemost*. 2017;117:1651–9.
[51] Mayer K, Hein-Rothweiler R, Schüpke S, Janisch M, Bernlochner I, Ndrepepa G, Sibbing D, Gori T, Borst O, Holdenrieder S, Kupka D, Petzold T, Bradaric C, Okrojek R, Leistner DM, Trippel TD, Münzel T, Landmesser U, Pieske B, Zeiher AM, et al. Efficacy and safety of revacept, a novel lesion-directed competitive antagonist to platelet flycoprotein VI, in patients undergoing elective percutaneous coronary intervention for stable ischemic heart disease: the randomized, double-blind, placebo-controlled ISAR-PLASTER phase 2 trial. *JAMA Cardiol*. 2021;6:753–61.

SUPPLEMENTARY MATERIAL

The online version contains supplementary material available at https://doi.org/10.1016/j.jtha.2023.07.018