Humanized Mouse Model of Thrombosis Is Predictive of the Clinical Efficacy of Antiplatelet Agents

Jorge Magallon, MD; Jianchun Chen, PhD; Leroy Rabbani, MD; George Dangas, MD, PhD; Jing Yang, PhD; James Bussel, MD; Thomas Diacovo, MD

Background—In vivo testing of novel antiplatelet agents requires informative biomarkers. By genetically modifying mouse von Willebrand factor (VWF<sub>R1326H</sub>), we have developed a small animal model that supports human but not mouse platelet-mediated thrombosis. Here, we evaluate the use of this biological platform as a pharmacodynamic biomarker for antithrombotic therapies.

Methods and Results—The antithrombotic effects of several αIbb3 inhibitors were determined in VWF<sub>R1326H</sub> mutant mice infused with human platelets. Administration of abciximab, eptifibatide, or tirofiban at doses recommended for percutaneous coronary intervention (per 1 kg of body weight) significantly reduced human platelet-mediated thrombus formation in laser-injured arterioles by >75% (P < 0.001). In contrast, clot size in wild-type control animals remained essentially unchanged (P > 0.05), results consistent with observed species differences in IC<sub>50</sub> values obtained by aggregometry. To further demonstrate that our biological platform is unique among standard mouse models, we evaluated the thrombogenic potential of platelets from healthy volunteers before and after clopidogrel therapy. Consistent with the antithrombotic effect of this agent, platelets postdrug administration formed smaller thrombi than cells before therapy and were less responsive to ADP-induced aggregation (P < 0.001).

Conclusions—The ability of αIbb3 and P2Y<sub>12</sub> inhibitors to limit human platelet clot formation at doses recommended by the American College of Cardiology/American Heart Association suggests that VWF<sub>R1326H</sub> mutant mice can serve as both a pharmacodynamic and a functional response biomarker, attributes essential for not only expediting drug development but also designing clinical studies. (Circulation. 2011;123:319-326.)

Key Words: cell adhesion molecules • drugs • platelets • thrombosis • von Willebrand factor

A rterial thrombosis is a central pathological mechanism contributing to myocardial infarction and stroke. A critical event in this process is the exposure of plaque contents to the intravascular environment, triggering platelet deposition and ultimately thrombus formation through a well-orchestrated series of adhesive and cell signaling events.1-3 For instance, the ability of circulating platelets to rapidly attach to an area of vascular damage is dependent on von Willebrand factor (VWF), a multidomain, multimeric glycoprotein that forms an adhesive bridge between platelets and exposed extracellular matrix proteins.4 In particular, the A1 domain of VWF initiates platelet adhesion by serving as a binding site for the platelet receptor glycoprotein Ibα (GPIbα).5,6 Stable adhesion and thrombus growth, on the other hand, require conversion of integrins such as αIbb3 and αIbb3 from an inactive to an active conformation so that platelets can adhere firmly to collagen and fibrinogen, respectively.7 This event relies on the activation of intracellular signaling pathways triggered by platelet interactions with the exposed extracellular matrix proteins or in respond to agonists released (ie, ADP and thromboxane A<sub>2</sub>) or generated (ie, thrombin) at the site of injury.8,9 Consequently, these mediators amplify and sustain integrin reactivity, leading to the further accrual of platelets and ultimately the formation of an occlusive clot.

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Detailed knowledge of these adhesive and activation events has led to the development of antiplatelet agents that are used to prevent and/or reduce arterial thrombosis. These include both oral inhibitors directed against the ADP receptor P2Y<sub>12</sub> (ie, clopidogrel, prasugrel, and ticagrelor) and short-term—use intravenous agents that prevent αIbb3-mediated platelet aggregation (ie, abciximab, tirofiban, and eptifibatide).10-13 Although considerable progress has been made over the past decade, a need still exists for more efficacious and safer antiplatelet drugs as the heart disease pandemic...
continues to increase. Clearly, the development of biological platforms that better reflect the intravascular environment in humans could expedite this process.

Animal models have been instrumental in broadening our understanding of platelet physiology and pathology and in drug development and testing. Moreover, they have been used to define criteria for therapeutic blockade in assay systems such as optical aggregometry. However, there are several limitations. For instance, studies in nonhuman primates are constrained by high cost, limited availability, and paucity of genetic models for human diseases. Rodent models avoid many of these issues, but experiments performed solely in these animals may not accurately predict outcomes in patients; differences in the structure and/or isoforms of proteins expressed on mouse versus human platelets may preclude in vivo testing. Thus, the creation of mice that carry partial or complete human physiological systems may help overcome these species differences.

Until recently, it was not possible to study human platelet-mediated thrombosis in mice because of impaired interactions with the injured vessel wall. By performing detailed structure/function analyses, we discovered that the primary defect was related to the reduced capacity of human GPIbα to interact with mouse VWF-A1. By genetically modifying the A1 domain in mice so that it more closely resembles its human counterpart, not only were human platelets able to form occlusive clots, but also, mouse platelets had a limited capacity to participate in this process. However, it remains to be shown whether this novel biological platform is truly superior to conventional mouse models in evaluating the in vivo efficacy of antiplatelet therapies. We therefore designed the present study to assess the effect of several αIIbβ3 inhibitors on human versus mouse platelet-mediated thrombosis in response to laser-induced vascular injury. Results indicate that VWF mutant animals, but not wild-type (WT) controls, can accurately predict the in vivo efficacy of such agents used at doses recommended by the American College of Cardiology/American Heart Association (ACC/AHA) for percutaneous coronary intervention (PCI).

Importantly, adhesion and signaling pathways critical for thrombus formation in humans were also required for this process in our animal model.

Methods

Antibodies and Reagents

Protease activated receptor (PAR)-1 (SFLLRN) and PAR-4 (AYPGKF) agonists were obtained from Bachem Bioscience (King of Prussia, PA). ADP and human and mouse fibrinogen were purchased from Sigma Co (Saint Louis, MO), Epitifactide (Integrilin 2 mg/mL) and clopidogrel (Plavix 75 mg) were obtained from the hospital pharmacy. Tirofiban (Aggrastat 250 μg/mL) and monoclonal antibody 6D1 (function-blocking antibody to human GPIbα) were kindly provided by Barry Coller (Rockefeller University, New York, NY). Abciximab (Reopro 2 mg/mL) was purchased from Centocor, Inc (Marvin, PA), XP280, an active metabolite of roxifiban, was provided by Bristol-Myers Squibb (Pennington, NJ).

Mice

VWFPR1326H mutant animals and WT littermates, both on a 129/SvJ background, were generated as previously described. Animal Care and Use Committees at Columbia University Medical Center.

Blood Collection

For studies involving human platelets, blood was obtained from healthy adult volunteers by drawing it into a syringe containing 3.8% trisodium citrate as anticoagulant. To determine the in vivo efficacy of clopidogrel, blood was drawn from these same individuals before and 8 hours after a single dose of the drug (300 mg). In every case, informed consent was obtained before blood draws and drug administration using a protocol approved by the institutional review committee at Columbia University Medical Center.

For studies evaluating the contribution of platelet dense granules or the integrin αIIbβ3 in human platelet-mediated thrombosis, blood was obtained from individuals with either Hermansky-Pudlak syndrome or Glanzmann thrombasthenia, respectively. In the former case, these individuals were of Cuban descent and had a 16-bp duplication in the HPS1 gene; the latter lacked expression of αIIbβ3 on the surface of their platelets. For studies involving mouse platelets, blood was obtained from anesthetized animals via cardiac puncture by drawing it into a syringe containing 3.8% trisodium citrate. Generation of plasma-rich protein or purified platelets was performed by centrifugation as previously described.

Platelet Aggregation

Blood was obtained from drug-treated or untreated humans and mice, and platelets were purified from plasma-rich protein by centrifugation. Cells were resuspended to a final concentration of 350 000/μL in buffer containing 145 mmol/L NaCl, 10 mmol/L HEPES, 0.5 mmol/L Na2HPO4, 5 mmol/L KC1, 2 mmol/L MgCl2, 1 mmol/L CaCl2, and 0.1% glucose, pH 7.4. Stock solutions of αIIbβ3 antagonist were prepared on the day of experimentation and added to platelet suspensions 5 minutes (37°C, 1200 rpm) before the induction of aggregation with ADP (20 μmol/L), PAR-1 agonist (25 μmol/L), or PAR-4 agonist (1 mmol/L). Human or mouse fibrinogen (final concentration, 200 μg/mL) was added to the platelet suspensions just before platelet activation. Aggregation was assessed with a Chronolog Lumi-Aggregometer (model 540 VS; Chronolog, Havertown, PA) and permitted to proceed for 6 minutes after the addition of agonist. The results are reported as maximum percent change in light transmittance from baseline with platelet buffer used as a reference.

Circulating Levels of Human Platelets in Mice

VWFPR1326H mutant mice (12 weeks of age; average weight, ~25 g) were depleted of endogenous platelets by administration of antibodies that react specifically with mouse GPIbα (Emfret Analyticals, Wurzburg, Germany). After confirming >85% reduction in platelet count 24 hours after administration, purified human cells (700K/μL) were infused at 25 μL/min for 15 minutes (1 mL BD syringe, PHD 2000, Harvard Apparatus Inc, Holliston, MA) through a catheter placed in the femoral artery. Human platelet counts were obtained at 2 and 10 minutes during the infusion by drawing blood from a catheter inserted into the internal jugular vein (Hemavet 950FS; Drew Scientific, Oxford, CT).

In Vivo Thrombus Formation

Administration of anesthesia, insertion of venous and arterial catheters, fluorescent labeling of human platelets, and surgical preparation of the cremaster muscle in 12-week-old male mice have been previously described. Injury to the vessel wall of arterioles (40- to 65-μm diameter) was performed with a pulsed nitrogen dye laser applied through a ×20 water-immersion Olympus objective. Human or mouse platelet–vessel wall interactions were visualized by fluorescence microscopy using a system equipped with a Yokogawa CSU-22 spinning disk confocal scanner, iXON EM camera, and 488- and 561-nm laser lines (Revolution XD, Andor Technology, South Windsor, CT) to detect BCECF- and rhodamine-labeled cells, respectively. The extent of thrombus formation was assessed for 2 minutes after injury, and the maximal area (μm2) of coverage was
determined by offline analysis (ImagePro Plus, Media Cybernetics, Inc, Bethesda, MD). For αIIbβ3 inhibition studies, abiximab, epifibatide, tirofiban, or XP 280 was first given as an intravenous bolus and then as a continuous infusion as recommended by the ACC/AHA for PCI.22 Human platelets (700 000/μL) were continuously infused (25 μL/min) through a catheter placed in the ipsilateral femoral artery 2 minutes before and during laser-induced injury to ensure a constant level of circulating cells equivalent to human. The infusion was continued for the duration of the experiment (~15 minutes). Four to 5 mice (minimum of 6 arteriole segments per mouse) were used per control or experimental condition.

Statistics
To compare mean thrombus areas between different treatment groups, we fit linear mixed models with random intercepts for each study animal. Linear mixed models permit comparison of mean differences between treatment groups while also considering the effect of the clustering of the data on the SEs resulting from multiple measurements obtained from each mouse used in intravital studies.27 In the case of platelet aggregation studies, values are presented as mean±SEM. A 2-tailed Student t test was used for comparisons between control conditions and treatments. Differences with values of \( P<0.05 \) were considered statistically significant.

Results
Adhesion and Activation Pathways Supporting Human Platelet Thrombus Formation
Previously, we reported that the R1326H mutation in the A1 domain of mouse VWF enables human platelets to preferentially support in vivo thrombus formation by preventing an unfavorable electrostatic interaction with human GPIbα (Figure 1A).20 Although we established the importance of the GPIbα–VWF-A1 axis in this biological platform, it remains to be determined whether downstream adhesion and activation events critical for this process in humans such as release of dense granule contents and subsequent activation of αIIbβ3 also contribute to thrombus growth and stability. To this end, we evaluated the ability of platelets obtained from individuals with either Hermansky-Pudlak syndrome (lack dense granules) or Glanzmann thrombasthenia (lack αIIbβ3) to form thrombi in laser-injured arterioles in the cremaster muscle of VWFR1326H mutant animals. Fluorescently labeled platelets from these individuals were continuously infused retrogradely through the femoral artery of VWF\(^{R1326H}\) mice to achieve and maintain a circulating platelet count that approximated those found in healthy individuals (Figure 1B). Consistent with the known defects in hemostasis, platelets from these individuals failed to support significant thrombus formation in response to vascular injury (Figure 1C). Compared with platelets from healthy volunteers, maximal thrombus size for platelets from Hermansky-Pudlak or Glanzmann patients was reduced by \( \approx 76\% \) and \( \approx 81\% \), respectively (\( P<0.001 \); see Table I in the online-only Data Supplement). In addition, blockade of GPIbα function significantly impaired thrombus formation by \( >95\% \) (\( P<0.001 \)), demonstrating that at least 3 key events known to contribute to platelet adhesion and aggregation are required for human thrombus formation in these animals.

Thrombotic Potential of Human Platelets Exposed to Clopidogrel
Because dense granules (a source of ADP) are essential for human thrombus formation in VWF\(^{R1326H}\) mutant animals, it is reasonable to assume that agents that prevent ADP-mediated platelet activation would also limit this process. To address this clinically relevant issue and to determine whether our biological platform may serve as a functional response biomarker, we studied the thrombotic potential of platelets purified from 6 healthy volunteers before and after a single dose of clopidogrel (300 mg). P2Y\(_{12}\) inhibition 8 hours after drug administration was confirmed by optical aggregometry, which yielded a reduction in ADP-induced aggregation that ranged from 36% to 84% (combined mean value, 57% for all 6 individuals; Figure 2A).28 Consistent with these results are the in vivo observations that human platelet-mediated thrombus formation was curtailed to a similar degree in response to clopidogrel, which ranged from 32% to 77% (combined mean value, 63% for all 6 individuals; Figure 2B; see Table II in the online-only Data Supplement). Importantly, there is a trend suggesting that the degree of inhibition observed by optical aggregometry for each volunteer may parallel that observed for in vivo thrombus formation (\( r=0.77 \); Figure 2C).

In Vivo Efficacy of αIIbβ3 Inhibitors
We next set out to establish the potential pharmacological utility of VWF\(^{R1326H}\) mutant animals. Because the platelet receptor αIIbβ3 has been a pharmacological target of considerable interest, owing to its vital role in promoting platelet aggregation and thrombus stability,29–31 we evaluated the effects of 4 anti-αIIbβ3 agents on human versus mouse platelet-mediated thrombosis. Drug doses were based on guidelines established by the ACC/AHA for PCI except for XP280, an active metabolite of roxifiban, which was based on a previous study in canines.22 Results indicate that administration of abiximab, epifibatide, or tirofiban to WT animals

![Figure 1](https://example.com/figure1.png)
had no significant effect on mouse platelet-mediated thrombus formation (P > 0.05; Figure 3A and 3B; see Table III in the online-only Data Supplement). Only XP280 was able to limit this process, resulting in a reduction in maximal thrombus size by \( \frac{1}{1.5} \times 78\% \) compared with control (P < 0.001). In contrast, all 4 anti-\( \alpha_{IIb} \beta_3 \) agents curtailed human platelet-mediated thrombosis in VWF \( ^{R1326H} \) mutant animals by >75\%, with abciximab yielding the greatest overall reduction in size (Figure 3C and D; see Video I and Table IV in the online-only Data Supplement). In the case of XP280, thrombus size was reduced to a level observed in WT mice, suggesting that it is an effective \( \alpha_{IIb} \beta_3 \) blocker in both species.

**In Vitro Effect of \( \alpha_{IIb} \beta_3 \) Inhibitors on Human Versus Mouse Platelet Function**

To determine whether our in vivo observations are consistent with results obtained by in vitro analyses currently used to ascertain the potential effects of antiplatelet agents, we next compared the ability of abciximab, eptifibatide, tirofiban, and XP280 to disrupt ADP-induced aggregation of purified human versus mouse platelets in fibrinogen-supplemented buffer. Although the inhibitory effects of these agents on human platelet function were consistent with previous reports, \(^{32}\) their ability to prevent ADP-mediated mouse platelet aggregation was limited (Figure 4A through 4D). This is exemplified by observed differences in IC\( _{50} \) values obtained for eptifibatide and tirofiban, which were 25-fold and 148-fold greater for mouse platelets, respectively (Table). No inhibitory effect was observed for abciximab, and only XP280 yielded a similar degree of inhibition for both species as observed in vivo.

To further illustrate the differences in \( \alpha_{IIb} \beta_3 \) blockade, mouse platelets were exposed to concentrations of abciximab (3 \( \mu \)g/mL), eptifibatide (120 nmol/L), tirofiban (30 nmol/L), abciximab (3 \( \mu \)g/mL), eptifibatide (120 nmol/L), tirofiban (30 nmol/L), and XP280 to disrupt ADP-induced aggregation of purified human versus mouse platelets in fibrinogen-supplemented buffer. Although the inhibitory effects of these agents on human platelet function were consistent with previous reports, \(^{32}\) their ability to prevent ADP-mediated mouse platelet aggregation was limited (Figure 4A through 4D). This is exemplified by observed differences in IC\( _{50} \) values obtained for eptifibatide and tirofiban, which were 25-fold and 148-fold greater for mouse platelets, respectively (Table). No inhibitory effect was observed for abciximab, and only XP280 yielded a similar degree of inhibition for both species as observed in vivo.

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or XP280 (30 nmol/L) that completely blocked ADP-induced human platelet aggregation. As a result, <10% inhibition was achieved under identical conditions (Figure 5A through 5E). Only XP280 was able to reduce mouse platelet aggregation to a level observed for their human counterpart. Consistent with these observations were the findings that platelets harvested from WT mice treated with eptifibatide (180 μg/kg bolus) followed by a 10-minute infusion at 2 μg·kg⁻¹·min⁻¹ or tirofiban (25-μg/kg bolus followed by a 10-minute infusion at 0.15 μg·kg⁻¹·min⁻¹) aggregated to a degree similar to that of nontreated animals (51.6±4.8% and 46.3±2.9% versus 55±2.7%, respectively; P>0.05; Figure 5F). In contrast, platelets from WT mice that received XP280 (0.1-μg/kg bolus) failed to undergo ADP-induced aggregation (4.7±1.6%; P<0.001).

It is well known that the concentration of αIIbβ3 inhibitors required to prevent human platelet aggregation is agonist dependent, further complicating the determination of drug efficacy. We therefore compared the ability of anti-αIIbβ3 agents to prevent thrombin receptor-activating peptide-induced human or mouse platelet aggregate formation at a concentration of agonist that yielded maximal reactivity. Platelets were first incubated with a concentration of drug that blocked >90% of ADP-induced human platelet aggregation (Figure 6A through 6D). No matter which drug or species of platelets was used, the degree of inhibition never approached that observed in the presence ADP. In fact, the maximal achievable level of inhibition observed for thrombin receptor-activating peptide–induced aggregation was only 53%, which occurred in the presence of abciximab (Figure 6A). Even a ∼30-fold increase in the concentration of these αIIbβ3 blocker was not sufficient to completely abrogate platelet aggregation.

### Discussion

Mouse models of arterial thrombosis have played a valuable role in broadening our understanding of the molecular mechanisms that govern platelet adhesion and activation at sites of vascular injury. Consequently, it has been a long-held belief that these vascular injury models would faithfully reproduce the inhibitory effects of antiplatelet agents in humans. Contrary to this notion, we now demonstrate that there are considerable species differences in the ability of these drugs to alter platelet function both in vitro and in vivo. This was clearly evident for abciximab, eptifibatide, and tirofiban, all of which are currently used in clinical practice. Our unique biological platform, however, can circumvent this impediment to preclinical drug screening by permitting the in vivo testing of antithrombotic agents directly against the target cells of interest, human platelets, demonstrating its potential as a pharmacodynamic biomarker. Importantly, these Food and Drug Administration–approved inhibitors were effective in mitigating human platelet-mediated thrombus formation in VWFRIIa/3041 mutant mice at doses recommended by the ACC/AHA for PCI. This level of inhibition could not be achieved in either a vascular injury model or optical aggregometry with WT mouse platelets.

Mechanistically, the lack of effective blockade of thrombosis by abciximab, eptifibatide, and tirofiban indicates that structural differences in this integrin receptor may preclude the testing of these agents against mouse platelets. Indeed, this is supported by elegant structure/function analyses performed by several groups of investigators. For instance, abciximab is an Fab fragment of the monoclonal antibody 7E3 and is thought to impede fibrinogen interactions with αIIbβ3 through steric hindrance and/or by causing allosteric changes in its RGD binding region. Interestingly, it was determined that a difference in 4 key amino acids located within and adjacent to the specificity-determining loop of the folded protein accounted for its inability to bind to and block the function of mouse αIIbβ3. This would explain why abciximab is ineffective in preventing aggregation of mouse platelets in both in vitro and in vivo assays. Eptifibatide and tirofiban, on the other hand, are small molecules that mimic the RGD motif in fibrinogen and VWF and thus compete with this native ligand for binding to this integrin. Although all mammalian species rely on αIIbβ3 interactions for platelet aggregation, rodent platelets have been shown to be less responsive to inhibition with RGD-based compounds. This is thought to result from differences in residues within

### Table. Concentrations of Anti-αIIbβ3 Agents Required to Achieve 50% Inhibition of ADP-Induced Human Versus Mouse Platelet Aggregation

<table>
<thead>
<tr>
<th>Species (Platelets)</th>
<th>Abciximab, μg/mL</th>
<th>Eptifibatide, nmol/L</th>
<th>Tirofiban, nmol/L</th>
<th>XP 280, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>0.67±0.01</td>
<td>40±2.8</td>
<td>11.5±0.7</td>
<td>14.8±1.3</td>
</tr>
<tr>
<td>Mouse</td>
<td>None</td>
<td>1009±33*</td>
<td>1701±101*</td>
<td>21±3*</td>
</tr>
</tbody>
</table>

*P<0.01 versus untreated platelets (2-tailed Student t test).
the αIIb chain that comprise the β-propeller, a region that supports ligand binding.\textsuperscript{38,39} Interestingly, only the active metabolite of roxifiban, which was also designed to mimic the RGD sequence in the fibrinogen, was able to inhibit platelet aggregation and thrombus formation to a similar degree in both species at nearly identical concentrations of drug. This suggests that roxifiban must interact with similar residues/structural features in the fibrinogen-binding region of both the human and mouse receptor. That said, the ability of this inhibitor to reduce human and mouse platelet-mediated thrombosis to a similar extent suggests that αIIbβ3 on human platelets can effectively interact with ligands of mouse origin. This is consistent with in vitro studies demonstrating the ability of mouse fibrinogen and VWF to support human platelet firm adhesion in response to ADP stimulation (Figure I in the online-only Data Supplement).

Although results demonstrate the utility of our biological platform in determining the in vivo efficacy of αIIbβ3 inhibitors, this is only one of several classes of antiplatelet drugs. Another pharmaceutical target of considerable interest is the ADP receptor P2Y\textsubscript{12}. Currently, P2Y\textsubscript{12} antagonists combined with aspirin therapy are the cornerstones in the management of acute coronary syndromes and in patients undergoing PCI.\textsuperscript{21,40,41} However, no consensus has been reached regarding the best technology to accurately predict the antithrombotic effect of these drugs in patients.\textsuperscript{42–44}

Because results clearly demonstrate that thrombosis in VWFR\textsuperscript{R1326H} mice is critically reliant on ADP release from and subsequent activation of human platelets, our biological platform may provide the in vivo correlation required to validate such monitoring devices by enabling the study of the platelets isolated from drug-treated individuals under physiologically relevant conditions. Thus, VWFR\textsuperscript{R1326H} mutant animals may serve as a functional response biomarker by permitting us to assess whether the antiplatelet agents have the intended biological response.

**Conclusions**

We demonstrate the pharmacological utility of a unique small animal model that permits the in vivo evaluation of human platelet interactions with the injured vessel wall. In particular, this biological platform can predict the in vivo response of antiplatelet drugs in humans more accurately than conventional mouse models of thrombosis, which would shorten drug development time, enable better determination of dosing regimens, and aid in the design of future clinical studies. The ability to assess the in vivo effectiveness of antiplatelet drugs...
administered directly to humans may also provide useful information for determining the in vitro relevance of proposed therapeutic indices established by current platelet function monitoring devices.32

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We would like to thank Roger Vaughan and Jimmy Duong from the Department of MSPH Biostatistics and Irving Institute for Clinical and Translational Research at Columbia University for their expert help in statistical analyses of the data.

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Disclosures
Dr Yang is employed by the company whose potential product was studied in the present work. The other authors report no conflicts.

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**CLINICAL PERSPECTIVE**

Platelet adhesion and activation are key events contributing to arterial thrombosis in disease states such as atherosclerosis, the leading cause of morbidity and mortality in industrialized countries. Although our current therapeutic armamentarium for preventing and treating complications associated with atherothrombosis has yielded promising results, there remains an urgent need for more effective drugs and better monitoring of treatment modalities. To expedite this process, it is necessary to develop animal models that more accurately recapitulate the human response to antiplatelet agents. In the present report, we demonstrate how mice bearing a genetically modified form of VWF can serve as a biological platform for predicting the in vivo efficacy of such therapies by enabling human platelet thrombus formation at sites of vascular injury, an event critically reliant on adhesion receptors, secretory products, and activation pathways known to contribute to this process in atherosclerotic coronary arteries (ie, αIIbβ3, ADP, and P2Y12). This is further supported by the ability of the Food and Drug Administration–approved αIIbβ3 blockers abciximab, eptifibatide, and tirofiban to effectively limit human but not mouse platelet-mediated clot formation at doses recommended by the American College of Cardiology/American Heart Association for percutaneous coronary intervention. However, the most beneficial aspect of this model may be its ability to ascertain the inhibitory capacity of antiplatelet agents after administration to patients. As such, it may aid in correlating values obtained by function monitoring devices with the thrombogenic capacity of platelets when exposed to sites of vascular damage in a living animal, thus providing new insights into this highly debated area of clinical research.
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Figure 1. Human platelet interactions with mouse fibrinogen and VWF. 

A, Flow chamber analysis evaluating the ability of ADP-stimulated human platelets to resist shear stress-induced detachment from surface-immobilized mouse versus human fibrinogen in the absence or presence of abciximab (2 independent experiments; n=4). B, The ability of soluble mouse versus human fibrinogen to support human platelet aggregation in response to ADP. Results are the mean ± SEM of 3 independent experiments performed on separate days. C, Flow chamber analysis evaluating the ability of ADP to induce firm adhesion of human platelets translocating
on either human or mouse plasma VWF in the absence or presence of abciximab (2 independent experiments performed in duplicate).

Table 1
Linear mixed model comparing average human platelet mediated thrombus formation (max. thrombus size in $\mu m^2$) in VWF$^{R1326H}$ mutant mice using healthy human platelets in the absence or presence of the GPIb$\alpha$ blocking antibody 6D1 or those lacking dense granules (Hermansky-Pudlak) or $\alpha$IIb$\beta$3 (Glanzmann). The reference group is healthy human platelets.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Regression coefficient</th>
<th>Standard error</th>
<th>$P$-value</th>
</tr>
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<tbody>
<tr>
<td>Intercept</td>
<td>8,390</td>
<td>189</td>
<td>&lt;0.001</td>
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<tr>
<td>mAb 6D1</td>
<td>-8,191</td>
<td>268</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hermansky-Pudlak</td>
<td>-6,390</td>
<td>268</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glanzmann</td>
<td>-6,785</td>
<td>268</td>
<td>&lt;0.001</td>
</tr>
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</table>

Table 2
Linear mixed model comparing average human platelet mediated thrombus formation (max. thrombus size in $\mu m^2$) in VWF$^{R1326H}$ mutant mice using platelets from healthy volunteers pre- and post-clopidogrel.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Regression coefficient</th>
<th>Standard error</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>6,841</td>
<td>353</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8h post treatment</td>
<td>-4,282</td>
<td>500</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 3
Linear mixed model comparing average mouse platelet mediated thrombus formation (max. thrombus size in $\mu m^2$) in WT mice in the absence or presence of one of the following drugs: abciximab, eptifibatide, tirofiban, or XP280. The reference group is the untreated animals.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Regression coefficient</th>
<th>Standard error</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>7,692</td>
<td>233</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abciximab</td>
<td>621</td>
<td>329</td>
<td>0.08</td>
</tr>
<tr>
<td>Eptifibatide</td>
<td>102</td>
<td>349</td>
<td>0.8</td>
</tr>
<tr>
<td>Tirofiban</td>
<td>-574</td>
<td>349</td>
<td>0.12</td>
</tr>
<tr>
<td>XP280</td>
<td>-6,024</td>
<td>329</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 4
Linear mixed model comparing average human platelet mediated thrombus formation (max. thrombus size in $\mu m^2$) in VWF$^{R1326H}$ mutant mice. The groups consist of untreated animals or those that received one of the following drugs: abciximab, eptifibatide, tirofiban, or XP280. The reference group is the untreated animals.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Regression coefficient</th>
<th>Standard error</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>8,555</td>
<td>158</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abciximab</td>
<td>-8,094</td>
<td>237</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Eptifibatide</td>
<td>-6,503</td>
<td>349</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tirofiban</td>
<td>-6,497</td>
<td>349</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>XP280</td>
<td>-6,974</td>
<td>329</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Video

The effect of eptifibatide on human versus mouse platelet thrombus formation in laser-injured arterioles contained within the cremaster muscle of a VWF$^{R1326H}$ mutant or WT mice, respectively.

Video 1 (far left) demonstrates the ability of fluorescently labeled human platelets to form occlusive thrombi in VWF$^{R1326H}$ mutant arterioles in the absence of drug. Video 2 (second from left) shows that eptifibatide administered per recommendations of ACC/AHA guidelines can prevent the formation of occlusive thrombi formed of human platelets in the same animal. Video 3 reveals the extent of mouse platelet mediated thrombus formation in WT animals in the absence of drug. Video 4 demonstrates that eptifibatide administered per recommendations of ACC/AHA guidelines does not have a significant effect on mouse platelet mediated thrombus formation in WT animals.