von Willebrand Factor
An Emerging Target in Stroke Therapy

Simon F. De Meyer, PhD; Guido Stoll, MD; Denisa D. Wagner, PhD*; Christoph Kleinschnitz, MD*

Background and Purpose—Thrombus formation is of paramount importance in the pathophysiology of acute ischemic stroke. Current antithrombotics used to treat or prevent cerebral ischemia are only moderately effective or bear an increased risk of severe bleeding. von Willebrand factor (VWF) has long been known to be a key player in thrombus formation at sites of vascular damage. While the association between VWF and coronary heart disease has been well studied, knowledge about the role of VWF in stroke is much more limited. However, in recent years, an increasing amount of clinical and preclinical evidence has revealed the critical involvement of VWF in stroke development.

Summary of Review—This review summarizes the latest insights into the pathophysiologic role of VWF-related processes in ischemic brain injury under experimental conditions and in humans. Potential clinical merits of novel inhibitors of VWF-mediated platelet adhesion and activation as powerful and safe tools to combat thromboembolic disorders including ischemic stroke are discussed.

Conclusions—Preclinical and clinical evidence illustrates an important role of VWF in ischemic stroke, suggesting that VWF could become a promising target in stroke therapy. (Stroke. 2012;43:00-00.)

Key Words: glycoprotein lb ■ platelets ■ stroke ■ von Willebrand factor

Ischemic stroke is a devastating disease that represents the primary reason for sustained disability and the second leading cause of death worldwide. Eighty percent of strokes are caused by arterial occlusion of cerebral arteries, whereas the remaining 20% are caused by intracerebral hemorrhages. Currently, the only established therapeutic option for acute stroke is rapid thrombolysis using the clot-breaking agent tissue-type plasminogen activator (tPA) to achieve recanalization of occluded cerebral vessels. However, due to the increased risk of bleeding associated with late tPA administration, intravenous tPA is recommended only within the limited therapeutic time window of 4.5 hours poststroke and thus is available to <10% of patients. A recent trial to extend the therapeutic window up to 9 hours by use of recombinant desmoteplase, a novel plasminogen activator, failed as did a trial using the defibrinogenating agent ancrod.

In terms of secondary stroke prevention, the situation is very similar. Antiplatelet agents such as acetylsalicylic acid, dipyridamole, and the platelet P2Y12 receptor inhibitor clopidogrel show only limited efficacy and substantially increase the risk of fatal bleeding. This holds also true for anticoagulants, particularly warfarin, and even the introduction of novel substance classes such as direct thrombin inhibitors (eg, dabigatran) or factor Xa blockers (eg, rivaroxaban, apixaban) could not overcome the threat of hemorrhage. These limitations emphasize the need for a better understanding of the pathophysiologic mechanisms of thrombus formation in acute ischemic stroke to successfully improve treatment.

von Willebrand Factor: Role in Hemostasis, Thrombosis, and Inflammation

In 1926, Finnish physician Erik von Willebrand reported a new type of inherited bleeding disorder that was distinct from hemophilia A. Thirty years later, the plasma protein that is central to the disease was identified and was named von Willebrand factor (VWF). Now, >50 years later, much of the structure and function of VWF has been elucidated and its role in maintaining the delicate balance between bleeding and thrombosis has become an intriguing subject. VWF is a large, multimeric glycoprotein. Along with serving as a protective carrier molecule for clotting factor VIII, its main function is mediating initial platelet adhesion at sites of vascular injury. Indeed, whereas this is a prerequisite for normal hemostasis, adhesion of platelets is also the first step in thrombosis and an important mediator of inflammation.
The critical role of vWF in normal hemostasis is exemplified by von Willebrand disease. von Willebrand disease is the most common inherited bleeding disorder in humans, caused by quantitative or qualitative defects in vWF. Bleeding symptoms range from mild (Type 1) to severe (Type 3) and include mucosal hemorrhages such as epistaxis, menorrhagia, and bleeding from the gums and gastrointestinal tract.

vWF is exclusively synthesized in endothelial cells and megakaryocytes and circulates as multimers of varying size (up to 20,000 kDa). The multidomain structure of the monomeric vWF building blocks (Figure 1) is fundamental to the function of vWF (Figure 2). Rapid binding of vWF (A3 domain) to exposed fibrillar collagen Type I and III immobilizes vWF at sites of vascular damage. This and/or high shear blood leads to conformational changes, exposing the binding site for platelet glycoprotein (GP)Ib in the vWF A1 domain. The reversible nature of the GPIb–vWF A1 interactions allows deceleration and rolling of platelets, which, especially under high shear forces, is necessary for the definitive arrest. Firm adhesion of platelets at the site of vascular injury is further supported by engagement of the platelet collagen receptors (GPVI and integrin αbβ3) and leads to platelet activation. On platelet activation, soluble platelet agonists such as adenosine 5'-diphosphate, adenosine 5'-triphosphate, and thromboxane A2 are released and platelet integrins like GPIIb/IIIa shift to a high-affinity state. Subsequent platelet aggregation is promoted by binding of activated platelet GPIIb/IIIa to its primary ligand fibrinogen and to the Arg-Gly-Asp sequence found in the C1 domain of vWF. Additional incoming platelets are recruited to the growing thrombus, primarily through engagement of their GPIb receptors (Figure 2). Hence, the GPIb complex is essential for both initial platelet adhesion to sites of vascular injury and recruitment of new platelets to the growing thrombus.

Besides its role in thrombus formation, vWF has also been shown to support inflammatory processes. In vitro experiments demonstrated that vWF promotes leukocyte adhe-
sion by acting as a ligand for the leukocyte receptors P-selectin glycoprotein ligand 1 and β2 integrin. vWF-bound platelets also were shown to support leukocyte tethering and rolling under high shear stress. More recently, Petri et al showed that vWF promotes the extravasation of leukocytes from blood vessels in a strictly platelet and GPIbα-dependent way.

ADAMTS13: A Biological Regulator of vWF Activity

vWF activity is correlated with multimer size, with ultralarge multimers spontaneously binding to platelets. Ultralarge vWF is released from endothelial and platelet storage granules on stimulation with various thrombogenic or inflammatory secretagogues, but also during hypoxia. To prevent spontaneous thrombosis, ADAMTS13 (a disintegrin and metalloprotease with thrombospondin Type 1 repeats 13) cleaves the Y1605-M1606 bond in the vWF A2 domain (Figures 1 and 2), thereby digesting ultralarge vWF into smaller, less reactive molecules. As seen in thrombotic thrombocytopenic purpura, ADAMTS13 deficiency is associated with thrombotic occlusion of microvessels from multiple organs, including the brain. Experimental studies in mice have demonstrated the antithrombotic and anti-inflammatory properties of ADAMTS13.

vWF and Stroke Risk

Because of the pivotal role of vWF in platelet adhesion and thrombus formation, it seems logical to anticipate a correlation between high plasma levels of vWF and development of cardiovascular disease. Although this relationship has been well studied for coronary heart disease, much less is known about the association between vWF levels and stroke. When comparing patients with stroke with healthy control subjects, several studies found an association between high vWF levels and stroke and even different etiologic subtypes of ischemic stroke. However, increased vWF levels are a well-known marker of endothelial activation and/or dysfunction and a conclusive interpretation of many of these case-control studies on the causative or consequential nature of high vWF levels in patients with stroke has been obscured by the poststroke time point of vWF measurement. By also measuring the vWF propeptide, a recent investigation of 2 independent case-control studies elegantly indicated that endothelial cell activation and subsequent Weibel-Palade body secretion is indeed an important mechanism underlying the association between total vWF levels and the occurrence of a first ischemic stroke. Several prospective studies such as the recent longitudinal analysis embedded in the Rotterdam study clearly identified high plasma levels of vWF as a strong predictor of stroke. Importantly, correlation between vWF levels and stroke-related mortality persisted after adjustment for common risk factors such as age, stroke severity, and atrial fibrillation indicating that vWF is an independent predictor. On a genetic level, vWF polymorphisms have been identified that significantly raise the risk of ischemic stroke. Not all of these are associated with higher vWF levels, suggesting that other mechanisms such as increased vWF activity could contribute to the risk of stroke as well.

A major downregulator of vWF activity is ADAMTS13. Accordingly, low levels of ADAMTS13 come along with an increased risk of cardiovascular disease, including ischemic stroke. Analogous to vWF, single nucleotide polymorphisms in ADAMTS13 were found to be associated with the incidence of ischemic stroke in a Swedish population.

Together, these proof-of-principle studies established that plasma levels of vWF and/or ADAMTS13 are associated with the risk of stroke in the general population. However, determination of vWF and/or ADAMTS13 levels on a regular basis to judge the risk of thromboembolic disease in individual patients cannot be recommended until larger prospective trials and standardized test systems are available.

Experimental Studies: Role of vWF in Acute Stroke

The importance of vWF as a risk factor for stroke occurrence and mortality in humans recently also stimulated experimental studies in models of acute stroke. Using a mouse model of transient middle cerebral artery occlusion, we showed that mice that are deficient in vWF due to vWF gene abrogation (VWF−/−) are protected from brain ischemia/reperfusion injury. Infract sizes in VWF−/− were approximately 60% of the infarct volumes in wild-type controls 1 day after transient middle cerebral artery occlusion, which was accompanied by reduced fibrin accumulation in the infarcted brain hemisphere. Accordingly, neurological scores assessing motor function and coordination were significantly better in VWF−/− mice compared with controls. Importantly, genetic disruption of vWF did not increase the risk of intracerebral bleeding in the context of ischemic stroke. Reconstitution of plasma vWF by hydrodynamic gene transfer fully restored the susceptibility of VWF−/− mice to cerebral ischemia underlying the causative role of vWF in this setting. This is in line with the well-established antithrombotic effects of vWF deficiency in several experimental arterial and venous thrombosis models. Further illustrating the critical role of vWF in ischemia/reperfusion injury are the findings that ADAMTS13−/− mice are more susceptible to focal cerebral ischemia. These mice developed significantly larger infarctions with an increased accumulation of immune cells and thrombi in the ischemic brain tissue, resulting in more severe neurological deficits. On the other hand, intravenous administration of recombinant ADAMTS13 into wild-type mice immediately before reperfusion significantly reduced infarct volume.

By reconstituting VWF−/− mice with different vWF mutants, we recently showed that binding of vWF to both collagen and GPIbα, but not to GPIIb/IIa, is a mandatory step in stroke development. The involvement of collagen and GPIbα-mediated platelet adhesion in stroke is corroborated by the findings that blocking platelet collagen receptor GPVI or GPIbα also confers a protective effect in the mouse transient middle cerebral artery occlusion model. Blockade of GPIIb/IIa did not affect stroke size and led to an increased incidence of intracerebral hemorrhage, whereas blocking of GPIbα or GPVI did not increase the frequency of intracere-
bral bleeding. Finally, mice in which downstream signaling of GPIb through phospholipase D1 is abrogated and mice in which the extracellular part of GPIb/H9251 is replaced by human interleukin-4 receptor (GPIb/H9251/IL4R/H9251) are also protected against focal cerebral ischemia without causing excessive bleeding. These observations further underline that blockage of the GPIb–vWF axis or collagen–platelet axis might be a safe approach in ischemic stroke.

Inhibitors of vWF: A Promising Class of Antithrombotics on the Brink of Reaching the Clinic

From this, it is clear that pharmacological interference in vWF-mediated platelet adhesion and thrombus formation could have clinical benefit as a promising strategy in stroke treatment. Although no such vWF blockers have yet achieved regulatory approval for marketing, there are promising preclinical and clinical studies that demonstrate the antithrombotic potential of agents that inhibit vWF function by blocking the vWF–collagen or vWF–GPIb/H9251 interaction (Figure 3).

In this section, we discuss candidate molecules that could prove useful in stroke therapy based on the encouraging results they have demonstrated in the inhibition of vWF-mediated thrombosis. These inhibitors include monoclonal antibodies against vWF (82D6A3, AJvW2 and its humanized form AJW200) or GPIbα (6B4 and its humanized form h6B4), the nanobody ALX-0081, the aptamer ARC1779, and the recombinant GPIbα fragment GPG-290 (Table). A detailed overview of the key features of each of these inhibitors is given in the online-only Supplemental Table (http://stroke.ahajournals.org).

Table. Inhibitors of vWF-Mediated Platelet Adhesion*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Description</th>
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<tbody>
<tr>
<td>82D6A3</td>
<td>Monoclonal antibody against vWF A3 domain that inhibits binding of vWF to collagen</td>
</tr>
<tr>
<td>6B4 h6B4</td>
<td>Fab-fragment of a monoclonal antibody against platelet GPIbα that inhibits binding of vWF to GPIbα</td>
</tr>
<tr>
<td>AJvW2 AJW200</td>
<td>Monoclonal antibody against vWF A1 domain that inhibits binding of vWF to GPIbα</td>
</tr>
<tr>
<td>GPG-290</td>
<td>Chimeric recombinant protein containing gain-of-function GPIbα fragment that binds to A1 domain thereby inhibiting binding of vWF to GPIbα</td>
</tr>
<tr>
<td>ARC1779</td>
<td>Aptamer against vWF A1 domain that inhibits binding of vWF to GPIbα</td>
</tr>
<tr>
<td>ALX-0081 ALX-0681</td>
<td>Nanobody against vWF A1 domain that inhibits binding of vWF to GPIbα</td>
</tr>
<tr>
<td>rADAMTS13</td>
<td>Recombinant protein that cleaves vWF multimers by cleaving the Y1605-M1606 bond in the vWF A2 domain</td>
</tr>
</tbody>
</table>

vWF indicates von Willebrand factor; GP, glycoprotein.
*A detailed description of each of these inhibitors is given in the online-only Supplemental Table (http://stroke.ahajournals.org).
AJW200 has been demonstrated in human volunteers without bleeding complications.58

GPG-290 is a homodimeric recombinant fragment of human GPIbα composed of the first 290 amino acids and conjugated to human IgG1Fc to form a homodimer. GPG-290 contains 2 gain-of-function mutations (G233V/M239V) resulting in its enhanced affinity for the vWF A1 domain (and possibly other GPIbα ligands). Its antithrombotic activity was demonstrated in preclinical murine and canine models of arterial and venous thrombosis.48,69,70

The aptamer ARC1779 represents an interesting new class of inhibitors. Aptamers are nucleic acid macromolecules that tightly bind to a specific molecular target.71 In solution, the chain of nucleotides forms intramolecular interactions that fold the molecule into a complex 3-dimensional shape that enhances affinity for its target molecule. A potential advantage of this class of molecules is that they can be inactivated by a complementary aptamer antidote if necessary. ARC1779 is a 40-nucleotide DNA/RNA aptamer conjugated to a 20-kDa polyethylene glycol to enhance its pharmacokinetic properties. ARC1779 binds to the vWF A1 domain and was reported to effectively inhibit thrombus formation in several preclinical settings.72,73 The aptamer was well tolerated in healthy volunteers in a Phase I trial. Importantly, in a Phase II trial, ARC1779 effectively increased platelet counts in critically ill patients with thrombotic thrombocytopenic purpura by blocking spontaneous vWF-mediated platelet aggregation74–76 and even prevented desmopressin-induced thrombocytopenia in patients with von Willebrand disease Type 2B.77 High-affinity aptamers to murine vWF have recently been developed, which will allow the investigation of anti-vWF aptamers in murine preclinical models of thrombosis, including stroke.78 Interestingly, a recent trial showed that ARC1779 was able to reduce cerebral emboli signals in patients undergoing carotid endarterectomy.79

Nanobodies are antibody-derived therapeutic proteins that contain the structural and functional properties of naturally occurring heavy-chain antibodies. The cameloid bivalent Nanobody ALX-0081 specifically targets exposed GPIbα-binding sites in vWF. By blocking GPIbα binding to vWF, ALX-0081 showed strong antithrombotic potency in preclinical baboon studies and in blood obtained from patients undergoing percutaneous coronary intervention.80,81 In a Phase I clinical study, this nanobody was found to be well tolerated and safe. ALX-0081 (and ALX-0681, a subcutaneous formulation of ALX-0081) entered a Phase II study in patients undergoing percutaneous coronary intervention and recently also a Phase II study in patients with thrombotic thrombocytopenic purpura. Apart from blocking binding of vWF to either collagen or GPIbα, another way of reducing vWF activity is decreasing its size by ADAMTS13. Recombinant ADAMTS13 is being developed as a new therapeutic agent and, as mentioned earlier, was protective in a preclinical mouse model of ischemic stroke.46 Recombinant human ADAMTS13 has received orphan designation for the treatment of thrombotic thrombocytopenic purpura (EU/3/08/588).

Potential Use of vWF Antagonism in Stroke Therapy

Because vWF antagonists are starting to enter the first clinical trials, it is interesting to speculate on the potential role of these new drugs in stroke therapy. Inhibitors of the vWF–GPIbα or vWF–collagen interaction have no direct thrombolytic properties, so their use is unlikely to result in dissolution of an already existing thrombus in the acute setting of ischemic stroke. However, when used in combination with tPA, vWF antagonists could prevent ongoing microvascular thrombus formation during the reperfusion phase after successful or spontaneous thrombolysis, reducing the occurrence of rethrombosis and/or secondary stroke progression. Whether dual therapy of vWF inhibitors and tPA would allow the use of lower and thereby safer doses of tPA remains to be established. The use of tPA-dependent experimental thromboembolic stroke models will have to shed more light on the possible beneficial effects of combining thrombolytic therapy with vWF inhibition. Former attempts to prevent the apposition of further clots into an already existing thrombus during the early stage of ischemic stroke by applying heparins failed.84 Although heparins could counteract deterioration of stroke symptoms in some studies, the net clinical benefit was outweighed by an increased risk of severe hemorrhages. Consequently, full-intensity parental anticoagulation with heparins is no longer recommended in the acute phase of cerebral ischemia.85 In view of the anticipated safer benefit-over-risk ratio in terms of bleeding, it will be interesting to see whether vWF antagonists are more successful than heparins during the acute phase of ischemic stroke. Nevertheless, close CT or MRI monitoring of potential hemorrhagic transformation should accompany potential future applications of vWF inhibitors in patients with stroke. Interestingly, recombinant ADAMTS13 promoted thrombus dissolution in injured mouse mesenteric arterioles,17 calling for further studies of the thrombolytic potential of ADAMTS13 in acute ischemic stroke.

With regard to stroke prevention, vWF inhibition could become useful for short-term therapy during procedures associated with increased risk of cerebral thromboembolism such as angiography, carotid endarterectomy, or heart surgery under conditions of extracorporeal circulation. Promising results were recently obtained with ARC1779, which significantly reduced cerebral embolization in patients undergoing carotid endarterectomy.79 In the absence of oral formulations, the potential of vWF blockers to prevent stroke or systemic embolism in chronic conditions, for example, in patients with atrial fibrillation, cannot yet be judged.

Conclusions

There is now compelling evidence on preclinical and clinical levels that interactions between the cerebral blood vessels and platelets using vWF, GPIbα, and collagen are instrumental in ischemic brain disease. Early clinical testing of lead compounds targeting vWF-mediated platelet adhesion in healthy individuals or selected disease states points toward a favorable bleeding risk profile and higher efficacy as compared with established antithrombotic drugs. Based on the encouraging results in rodent stroke models, larger translational
studies in nonhuman primates and proof-of-principle clinical trials are now warranted to further judge the risk-to-benefit ratio of interfering with the early steps of platelet activation. If these trials are as promising as the current experimental data, an exciting decade exploring vWF-targeted stroke strategies lies ahead. Back in 1926, Dr von Willebrand may not have fully grasped what great implications his seminal report⁶ on a bleeding disease would have on our understanding and treatment of thrombotic disorders. More than 90 years later, stroke may very well become one of them.

Acknowledgments
We thank Lesley Cowan for helping with the preparation of the article. We thank Dr Robert Schaub and Dr Karen Vanhoorelbeke for critical reading and helpful advice.

Sources of Funding
This work was supported by National Heart, Lung, and Blood Institute of the National Institutes of Health grant R01 HL041002 (to D.D.W.). S.F.D.M. is a postdoctoral fellow of the Research Foundation Flanders (Fonds voor Wetenschappelijk Onderzoek Vlaanderen), G.S. and C.K. are supported by the Deutsche Forschungsgemeinschaft, Bonn, Germany, SFB 688, projects A13 and B1.

Disclosures
None.

References


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Stroke. published online December 15, 2011;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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Supplemental Table. Inhibitors of VWF-mediated platelet adhesion

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<tr>
<th>Compound</th>
<th>Antithrombotic mechanism</th>
<th>Development stage</th>
<th>Preclinical and clinical antithrombotic evidence</th>
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<tr>
<td>82D6A3</td>
<td>Moab</td>
<td>preclinical</td>
<td>Epitope overlaps with collagen-binding region in VWF A3 domain¹</td>
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<td>Inhibited in vitro binding of VWF to collagen, with a more pronounced effect with increasing shear stress¹,²</td>
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<td></td>
<td>Interrupts collagen-VWF binding</td>
<td></td>
<td>Abolished thrombus formation in a thrombosis model in baboons without prolongation of bleeding time³</td>
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<td>Humanized version has been constructed⁴</td>
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<td>Moab (Fab)</td>
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<td>Epitope identified as two different regions in GPIbα⁵</td>
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<td>h6B4</td>
<td>Targets GPIbα</td>
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<td>Inhibited ex vivo VWF-mediated platelet agglutination and platelet adhesion to collagen⁶</td>
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<td></td>
<td>Interrupts VWF-GPIbα binding</td>
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<td>Fab fragments inhibited thrombus formation in two thrombosis models in baboons without significant effect on bleeding times⁶,⁷</td>
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<td>Fab fragments showed a broader safe therapeutic window compared to a</td>
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<td>AJvW2</td>
<td>AJW200</td>
<td>GPG-290</td>
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<td>Moab</td>
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<td>Conformation epitope identified in VWF A1 domain</td>
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<td>Inhibited ex vivo VWF-mediated platelet adhesion and agglutination</td>
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<tr>
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<td>Abolished thrombus formation and showed a superior bleeding safety profile in comparison to GPIIb/IIIa antagonism in both guinea pigs and dogs</td>
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<td>A humanized version (AJW200) was generated which inhibited thrombus formation in dogs with a safer bleeding profile compared to GPIIb/IIIa antagonism</td>
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<td>AJW200 was well tolerated in human volunteers without bleeding complications</td>
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<td>Chimeric recombinant protein containing gain-of-function GPIbα fragment</td>
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<td>Reduced venous thrombosis in mice</td>
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<td></td>
<td>Showed superior bleeding safety profile compared to clopidogrel</td>
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- Targets VWF A1 domain
- Interrupts VWF-GPIba binding

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<tr>
<th>ARC1779</th>
<th>Aptamer</th>
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<th>Inhibited VWF dependent platelet adhesion and agglutination(^\text{19})</th>
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<td>Inhibited thrombus formation in a cynomolgus monkey thrombosis model(^\text{19})</td>
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<td></td>
<td>Interrupts VWF-GPIba binding</td>
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<td>Well tolerated in human volunteers(^\text{20})</td>
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<td>Inhibited ex vivo VWF activity in blood from patients with acute coronary syndrome(^\text{21})</td>
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<td>Raised platelet counts in patients with TTP(^\text{22, 23})</td>
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<td></td>
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<td>Prevented thrombocytopenia in patients with VWD type 2B(^\text{24})</td>
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<td>reduced cerebral embolization in patients undergoing carotid endarterectomy(^\text{25})</td>
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| ALX-0081        | Nanobody\(^*\) clinical                     | Abolished thrombus formation in a baboon thrombosis model and showed superior therapeutic window compared to aspirin, clopidogrel and abciximab\(^\text{26}\) |
| ALX-0681        | Targets VWF A1 domain                        |                                              | Well tolerated and inhibited ex vivo platelet adhesion in patients having TTP or undergoing PCI\(^\text{26-28}\) |
|                 | Interrupts VWF-GPIba binding                 |                                              | Entered a Phase II study in TTP patients                            |

| rADAMTS13       | Recombinant protein preclinical               | Delays thrombus formation in a mouse thrombosis model\(^\text{29}\) |
- Cleaves VWF A2 domain
- Improves experimental stroke outcome in mice

Supplemental references


