proteins that interact with the SH3 domain of PSTPIP1 to create a broader picture of the general consequences of the R405C mutation.

Furthermore, the study by Starnes et al provides new insight into the role of PSTPIP1 as a regulator of the transition between podosomes and filopodia on 2D substrates. While there are examples of podosome alterations that correlate with inhibited ECM degradation, there is some evidence that podosome proteins accumulate along filopodia and matrix degrading activity in PSTPIP1-R405C cells than in control macrophages. This suggests that podosomes may be replaced by filopodia in pathologies, the ability of which to degrade ECM may be sufficient despite podosome disruption. It will be interesting to characterize whether filopodia-bearing cells in 2D do form 3D podosome structures in dense matrices.

Mutations in PSTPIP1 are found in patients with PAPA syndrome, which is caused by mutations in PSTPIP1. These mutations lead to a lack of podosome formation and an opposite phenotype, which would be expected if podosome formation is required for matrix degradation. However, in this study, the patient with the R405C mutation showed incomplete podosome disruption, indicating that PSTPIP1 may interact with other proteins to regulate podosome formation.

In conclusion, the study by Starnes et al provides a very important observation because it shows that the ability of this patient’s cells to migrate in dense matrices is highly efficient despite podosome disruption. It will be interesting to characterize whether filopodia-bearing cells in 2D do form 3D podosome structures in dense matrices.

In addition to their essential role in hemostasis, platelets play a crucial role in a pathological thrombus formation, particularly within atherosclerotic arteries subjected to high shear stress. As an initial step in thrombogenesis (or hemostasis), platelets adhere to altered vascular surfaces or exposed subendothelial matrices, then become activated and aggregate each other to grow a thrombus (or platelet plug) volume. These processes are mediated by the cytoskeletal proteins, talin and kindlin-3, to the platelet surface glycoproteins: GPIb-IX-V, integrin α3β1 (also known as GPIa-IIa), GPVI, and integrin αIIbβ3 (GPIIb-IIIa) integrins. Integrins play a family of αβ heterodimeric adhesion receptors that mediate cellular attachment to the extracellular matrix and cell cohesion. Among integrins, platelet αIIbβ3 is a prototypic non-I domain integrin and plays an essential role in platelet aggregation and thrombus growth as a physiological receptor for fibrinogen and von Willebrand factor, as evidenced by the clinical features of congenital bleeding disorder—Glanzmann thrombasthenia. After exposure to subendothelial matrix including collagen and von Willebrand factor and/or several mediators (agonists) including adenosine 5′-diphosphate (ADP), thromboxane A2, and thrombin, platelets become activated and a series of intracellular signaling events (“inside-out” signaling) that rapidly induce a high-affinity state of αIIbβ3 from its low-affinity state for soluble ligands (αIIbβ3 activation) are generated. In fact, αIIbβ3 activation is indispensable for platelet aggregation, and specific binding of the cytoskeletal proteins, talin and kindlin-3, to the cytoplasmic tail of the β3 subunit is the final common step in αIIbβ3 activation. Although αIIbβ3 represents a rationale target for antithrombotic therapy, strong inhibition of αIIbβ3 increased bleeding complication. This is also true in the case of P2Y12 inhibitors.
undermining hemostasis. In this issue of Blood, Stefani et al report a new strategy to segregate antithrombotic capacity from bleeding complications by modifying αIIbβ3 activation via disrupting of talin-1 interaction with β3 cytoplasmic tail. Talin-1 is a large protein (~270 kDa) that consists of a ~50 kDa globular head domain and an elongated flexible 220-kDa rod domain. Talin head domain contains 4 subdomains: F0, F1, F2, and F3, and recent structural and biochemical studies have established that αIIbβ3 activation requires interaction of talin F3 domain with 2 distinct binding sites in the β3 cytoplasmic tail: a membrane distal region (a proximal NPxY motif) and a MPR of β3, whereas kindlin-3 binds to the membrane-distal NxxY motif of the β3 cytoplasmic tail.

CMK, all talin mutants (L325R, S365D, S379R, and Q381V) selectively disrupting the binding to MPR completely lost their ability to activate αIIbβ3, whereas talin W359A retained some ability to activate αIIbβ3. To further elucidate the functional differences between talin L325R and talin W359A in vivo, the authors generate a series of platelet-specific talin-1 mutant knock-in mice. Because homozygotes for either talin-1 L325R or talin-1 W359A were embryonic lethal, talin-1 (L325R/wt) or talin-1 (W359A/wt) mice were crossed with platelet-specific talin-1 knockout (talin-1floxl/flox PFP4-Cre+) mice to generate compound heterozygous (talin-1 L325R/flox PFP4-Cre+) and control mice (talin-1wt/flox PFP4-Cre+). Thus, as compared with wild-type mice, these mice express only 50% of talin-1 in platelets, even in control mice (talin-1wt/flox PFP4-Cre+). The phenotype of talin-1 (L325R) mice was very similar to that of talin-1-deficient mice. These mice were protected from the experimental thrombosis experiments. It is especially noteworthy that talin-1 (W359A) mice were protected from the experimental thrombosis without pathological bleeding. Moreover, in vitro experiments revealed that talin-1 (W359A) head domain bound to β3 tail with 2.9-fold lower affinity compared with wild-type talin-1 head domain, which is likely responsible for slower αIIbβ3 activation, delayed platelet aggregation and markedly reduced ex vivo thrombus formation under high shear rate. However, under low shear rate, talin-1 (W359A) platelets had a capacity to form a small 3-dimensional thrombus.

This report reveals that the interaction of talin-1 with the MPR of β3 tail plays a more critical role in αIIbβ3 activation than with the NPxY motif in vivo. The disruption of the interaction with the NPxY motif did not abolish, but reduced, the affinity of talin-1 for β3 tail, thereby decelerating αIIbβ3 activation. It is likely that rapid αIIbβ3 activation on platelets is a prerequisite for pathological thrombus formation, particularly within atherosclerotic arteries subjected to high shear stress. This report newly proposes that deceleration of αIIbβ3 activation could be a smart way to prevent thrombosis without increasing bleeding tendency. Further studies are warranted to find the “magic bullet.”

Conflict-of-interest disclosure: The author declares no competing financial interests.
In this issue of Blood, Nguyen et al employ high-resolution mapping to precisely define epitopes on the C2 domain of blood coagulation factor VIII (FVIII). Their results nicely complement a recent report in Blood describing the structure of a ternary complex of 2 inhibitory antibodies with the C2 domain. Together, these studies reveal fascinating molecular details on the unexpectedly large number of exposed surfaces in the C2 domain that contribute to the binding of inhibitory antibodies. These findings are relevant in the context of neutralizing anti-FVIII antibodies that develop in patients with hemophilia A. Insight into the antigenic properties of the C2 domain is needed to design FVIII variants with decreased antigenicity. Pioneering studies by Dorothea Scandella on the epitope mapping of FVIII inhibitors already pointed toward the C2 domain as a major binding site for FVIII inhibitors. The recent studies by Nguyen and Walter, combined with earlier work by Meeks et al, have provided evidence for 3 major binding sites for inhibitory anti-FVIII antibodies within the C2 domain. The overall dimensions of the C2 domain are small when compared to antibodies. Nevertheless, at least 2 and probably 3 monoclonal antibodies can simultaneously bind to the C2 domain. The mapping studies reported by Nguyen also suggest the presence of 3 distinct clusters of surface-exposed side chains on the FVIII C2 domain (see figure).1

The results obtained raise the issue of why so many distinct antigenic sites are present within the C2 domain. The currently available structures of antibodies in complex with the C2 domain reveal that positively charged surfaces contribute to the binding of anti-C2 antibodies. These positively charged clusters contribute to the binding of FVIII to negatively charged phospholipids. Conversely, these patches of positively charged amino acids may also direct the immune response toward the C2 domain by promoting the selection of B-cell clones expressing antibody molecules with negatively charged residues in their variable domains.

It should be noted that the majority of monoclonal antibodies analyzed in this study are derived from hemophilia A mice injected with human FVIII. Epstein-Barr virus immortalization and phage display have been employed to isolate human monoclonal anti-C2 antibodies from peripheral blood of inhibitor patients. Reactivity of only a single human monoclonal antibody (BO2C11) belonging to the type AB group (see figure) was included in this study. Competition experiments have shown that so-called type BC/C antibodies (see figure) are also present in patients with FVIII inhibitors. Nevertheless, it would be important to extend the innovative studies reported in this article to a panel of human monoclonal antibodies derived from B cells of inhibitor patients.

In their search for antigenic sites on the C2 domain, Nguyen et al focused on antigenic variants that increased the dissociation rate of antibody–C2 domain complexes. This elegant approach has proven to be highly diagnostic for the identification of residues crucial for the high-affinity binding of antibodies to the C2 domain. Modification of the identified C2 domain residues in conjunction with antigenic loops in other antigenic sites within other domains provides an interesting approach for the development of less-antigenic variants of FVIII.

Apart from the proposed modification of B-cell epitopes on FVIII, a number of other approaches are currently being explored to prevent formation or eradicate preexisting inhibitors in hemophilia A patients. These efforts, together with the novel half-life-extending bioengineered FVIII molecules and the recent revival of gene therapy approaches, provide exciting new opportunities to further extend the current portfolio of therapeutic options for hemophilia A.

**Conflict-of-interest disclosure:** The authors declare no competing financial interests.

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Eliminate dark side from antiplatelet therapy

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