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Blocking direct inhibitor bleeding

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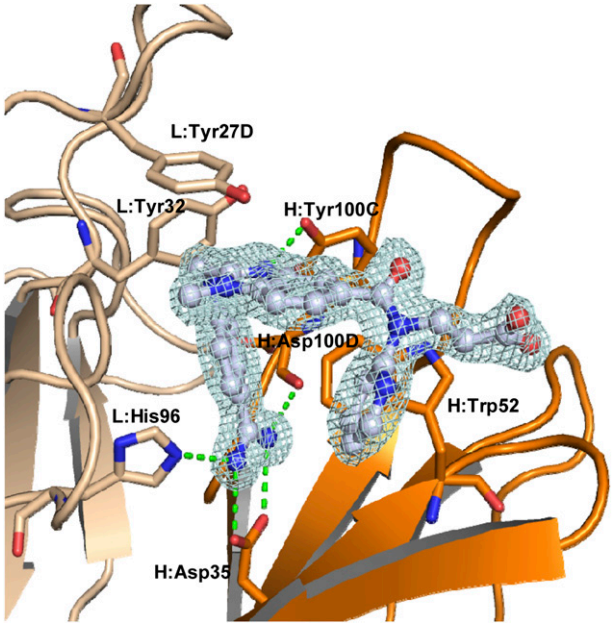
In this issue of *Blood*, Schiele et al report the development of a monoclonal antibody that reverses the anticoagulant effect of the direct thrombin inhibitor dabigatran.[1]

Novel oral anticoagulant agents that directly inhibit thrombin (dabigatran) or factor Xa (rivaroxaban, apixaban) represent a potential advance in the quest to replace vitamin K antagonists (VKAs) as the mainstay of oral anticoagulant therapy.[2] These agents may be equally if not more effective than VKAs in a number of clinical settings, while reducing the associated risk of intracranial hemorrhage. This, together with their shorter half-lives, fewer drug interactions, and more predictable pharmacokinetics, is likely to reduce their need for reversal when compared with VKAs. However, appropriate patient selection, as was required for their pivotal clinical trials, is paramount in clearly deriving the advantages of these novel agents. The absence of monitoring, as well as the heavy renal dependence of dabigatran and rivaroxaban for their elimination, suggest that there may be situations where control of therapy may be compromised. This was highlighted by our own recent case of an elderly female patient with renal impairment who sustained significant head injuries and loss of consciousness following a fall while intoxicated with alcohol. A supply of dabigatran was found in her coat pocket. A consequent intracerebral hemorrhage necessitated emergency neurosurgical decompression. Options for hemostatic support were limited to coagulation factor replacement therapy and prohemostatic agents. There is yet a paucity of clinical experience regarding the effectiveness and implementation of such treatments as well as important concerns regarding their associated prothrombotic risks. However, it is possible that such challenges posed by the use of dabigatran may be surmountable in the future following the report of an antidote for the reversal of its anticoagulant effect in this issue of *Blood* by Schiele et al.[1] They show that a customized monoclonal antibody can effectively inhibit dabigatran activity both in vitro and in vivo.



Dabigatran binding to the neutralizing monoclonal antibody, aDabi-Fab. See Figure 2 in the article by Schiele et al that begins on page 3554.

Specific therapeutic anticoagulant agent neutralization has distinct advantages over supportive therapy with activated coagulation factors or coagulation factor concentrates. Various strategies have been adopted to neutralize indirect and direct new anticoagulants, including attachment of a tag to the agent and the preparation of a recombinant, inactive, target protease. The former method (biotinylation) was used to provide a means of rapid binding and removal of the indirect factor Xa inhibitor, idraparinux,[3] from the circulation. The latter method was used by Lu et al,[4] who synthesized a truncated and inactive form of factor Xa that acted as a decoy and was able to reverse the anticoagulant effect of both direct and indirect inhibitors of factor Xa. Schiele et al[1] have instead used a monoclonal antibody approach, which has been successfully used in the neutralization of non-anticoagulant drugs, including digoxin, colchicine, and desipramine. They have generated the antibody, termed aDabi-Fab, from a hapten-immunogen derived from dabigatran. This was humanized, also allowing selection of higher affinity clones, which resulted in a binding affinity of ~2 pM, a 350-fold tighter binding than that of thrombin to dabigatran. The authors were able to show by x-ray crystallography that aDabi-Fab shares some structural features with thrombin that contribute to its high affinity molecular recognition of dabigatran. The benzamidine group of dabigatran was shown to be a principal mediator of this high-affinity interaction with aDabi-Fab, inserting into a cavity of the antibody binding site, forming a salt bridge with its heavy chain (H) residue Asp35 and important hydrogen bonds with its light chain (L) residue His96 and to H residue Asp100D (see figure).

This is similar to the way in which the benzamidine group of dabigatran binds to and blocks the S1 pocket of thrombin, interacting through a salt bridge with Asp189 at the bottom of the pocket and forming hydrogen bonds with residues Phe227 and Gly219 of thrombin. Moreover, the benzimidazole group and pyridine ring of dabigatran both participate in π-stacking with L Tyr27D and with H Trp52, similar to thrombin

interactions with Trp60D and Trp215, respectively. These structural similarities raised concern that aDabi-Fab may interact adversely with some of thrombin's many substrates. Although the aDabi-Fab lacks proteolytic machinery, it might contain some structural elements that could, for example, compete in the exosite reactions of the protease so important for its function.[5] Unwanted anticoagulant or procoagulant activities of aDabi-Fab could compromise its safety in clinical use. It was therefore important to eliminate the possibility of these secondary interactions by demonstrating absence of binding of aDabi-Fab to physiological substrates of thrombin using surface plasmon resonance. Likewise, aDabi-Fab was shown to not directly influence platelet aggregation. Thus, aDabi-Fab would appear to competitively inhibit the binding of thrombin to dabigatran, while avoiding mimicking any of the exosite driven activities of thrombin. Schiele et al[1] further supported this contention by demonstrating with thrombin-dependent in vitro functional clotting assays that aDabi-Fab is not active in the absence of dabigatran. Finally, it was shown that aDabi-Fab was also an effective in vivo antidote of dabigatran anticoagulant activity in rats, in which rapid reversal of anticoagulant effect in clotting assays was demonstrated.

Crucial to the success of this antidote to dabigatran will be additional studies of anticoagulant reversal in animal models of bleeding and, ultimately, in clinical investigation and trials of humans. In the meantime, this study does represent an important step in the development and use of the new oral anticoagulant agents. It shows that provision of a specific antidote is feasible and therefore suggests an effective means of controlling the anticoagulants in unpredictable clinical situations. If ultimately proven safe in human use, this will resolve what has until now been a pivotal limitation of these agents and provide an effective and safe strategy for situations in which immediate reversal of the anticoagulant effect is required.

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

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Pharmacotherapy versus T lymphocytes for CMV

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In this issue of *Blood*, Blyth et al report a phase 2 study in 50 allogeneic hematopoietic stem cell transplant (HSCT) recipients who received donor-derived cytomegalovirus (CMV)–specific cytotoxic T cells (CTLs) and compare outcomes with a group of concomitant controls who were transplanted at the trial centers but who did not receive CTLs.[1]

They show no significant difference in the incidence of acute or chronic graft-versus-host disease (GVHD) between groups or in the rate of CMV reactivation, but they observed a significant reduction in the percentage of patients who required CMV-directed antiviral therapy and in the total number of treatment days per patient in the cohort receiving CTLs. Notably, administration of virus-specific T cells did not induce acute or chronic GVHD, confirming other reports that virus-specific CTLs are not functionally alloreactive in vivo.[2] The authors therefore conclude that donor-derived CMV-specific T cells reduce the requirement for CMV-directed pharmacotherapy after allogeneic stem cell transplantation. Although their conclusions are limited by their nonrandomized trial design, this is nonetheless the first publication to compare outcome in patients who received donor CMV CTLs with a control group.

It has been 20 years since the first reports showed that donor-derived CMV-specific CTLs reconstituted protective donor immunity to CMV after allogeneic stem cell transplantation.[3] These observations have since been confirmed in a number of studies that include several hundred patients,[4,5] which justifies definitive testing in late-phase randomized trials. The report from Blyth et al, however, suggests that CMV reactivation rate should not be the end point for such studies because they observed no significant difference between CTL recipients and controls. This finding is expected, because even normal seropositive individuals have viral reactivations that are controlled by expansion of memory T cells. The investigators instead observed a difference in the requirement for CMV-directed antiviral therapy, since a higher percentage of patients who did not receive CTLs eventually had to receive such therapy and for a longer median duration than those who received CTLs. One limitation in the Blyth et al study is that decisions on starting and stopping antiviral therapy were made by the individual clinicians on the basis of their standards of practice, illustrating the importance of careful design of future randomized trials to ensure that such decisions follow a standardized procedure. It will also be beneficial to design future randomized studies to include comparative effectiveness analyses so the effect of CTLs on overall cost of antiviral and supportive therapy (including granulocyte colony-stimulating factor for drug-induced neutropenia) can be measured.

Before large-scale pivotal clinical studies of CTLs can be initiated, it will be necessary to simplify the manufacturing process to eliminate the requirement for viruses during production. Blyth et el initially used dendritic cells pulsed with an HLA2-restricted immunodominant peptide NLV from the CMV pp65 antigen to stimulate CTLs but then transitioned their