Xa and IX mark the clot

By Kai-Jye Lou, Staff Writer

Two research teams have separately reported engineered variants of clotting factors Xa and IX that could become next-generation hemophilia therapies.^{1,2} **Pfizer Inc.** has exclusively licensed the factor Xa variant, while an undisclosed company has licensed the modified factor IX.

In one of the studies, researchers at **The Children's Hospital of Philadelphia** and Pfizer showed that a mutant variant of human factor Xa stopped acute bleeding in mouse models of hemophilia. In the second study, a group led by researchers at the **German Red Cross Blood Donor Service Baden-Wuerttemberg-Hessen** designed a recombinant human factor IX variant that decreased bleeding times in mice.

Hemophilia is primarily caused by mutations in one of two clotting factor proteins—factor VIII and factor IX—and can result in severe and uncontrollable bleeding following injury. The condition is treated and prevented by injecting recombinant versions of factor VIII or factor IX. In patients refractory to those therapies, alternative clotting therapies such as factor VII are used.

C conspicuously absent from the list of approved factors is factor Xa, a serine protease that catalyzes one of the final steps in the clotting cascade—conversion of prothrombin to thrombin (factor IIa; F2).

Previous studies showed that factor Xa is rapidly inactivated by circulating serine protease inhibitors, resulting in a serum half-life of less than two minutes.³ Delivering high doses of the protein to offset the short half-life could lead to pathological clotting events.

In 2008, a group at the Children's Hospital of Philadelphia led by Rodney Camire reported the generation of mutant forms of factor Xa.⁴ The researchers were using the variants to explore the factor's transition from its inactive zymogen state to its activated protease state.

One of the mutants—factor Xa^{116L}—retained the ability to catalyze thrombin formation and was much better protected from inactivation by serum protease inhibitors compared with the wild-type protein.

Camire, an associate professor of pediatrics at the hospital and at the **Perelman School of Medicine at the University of Pennsylvania**, hypothesized that the factor Xa^{116L} variant could be useful for treating hemophilia. He teamed up with Pfizer scientists to evaluate factor Xa^{116L} in animal models of the disease.

In mouse models of hemophilia A and B, human factor Xa^{116L} showed a 30- to 60-fold increase in half-life and lowered clotting times compared with wild-type factor Xa. The molecule also decreased blood loss from a tail injury.

Also, human factor Xa^{116L} induced clot formation following injury at 25- to 45-fold lower doses than human factor VIIa. There was no evidence of spontaneous pathological clot formation in wild-type mice receiving daily high-dose injections of factor Xa^{116L} for three days.

Pfizer declined to comment on the study, which was published in *Nature Biotechnology*.

Corresponding author Camire expects factor Xa^{116L} will have the most benefit in a subpopulation of hemophiliacs with neutralizing antibodies to standard therapies. These patients rely on alternative clotting therapies such as NovoSeven, a recombinant human coagulation factor VIIa from **Novo Nordisk A/S**.

"NovoSeven works great but patients need to be given multiple large doses, which substantially increases treatment times and cost. Our mouse data suggest that our factor Xa variant is more potent and works faster than NovoSeven. We think this is because factor Xa acts further downstream than NovoSeven in the coagulation cascade and closer to the generation of thrombin," said Camire.

Factor Xa^{116L} also could be useful for treating acute bleeding episodes in the broader hemophilia patient population.

"Because factor Xa^{116L} acts near the end of the coagulation cascade, the enzyme also could be considered for use in more general acute bleeding settings, such as in cases of trauma and severe bleeding," said Jörg Schüttrumpf, research group leader and head of the production department at the German Red Cross Blood Donor Service Baden-Wuerttemberg-Hessen.

Schüttrumpf said the current form of factor Xa^{116L} still has a half-life that would make it impractical to use in a chronic prophylactic setting to help maintain clotting ability in hemophiliacs. "The engineered factor Xa is very potent and would most likely be used in an acute bleeding setting," he told *SciBX*.

Camire and colleagues previously reported that factor Xa^{116L} has a half-life of about 60 minutes in hemophilic plasma.³

Chronic factors

In October, Schüttrumpf's group, along with the lead author of the *Nature Biotechnology* paper, published a study describing an engineered variant of factor IX that could be more suitable for prophylactic use in hemophiliacs than factor Xa^{116L}.

"These two molecules would most likely be used in complementary settings," he said.

Schüttrumpf's group introduced three amino acid substitutions into factor IX to increase its enzymatic activity in the absence of factor VIII, which is required for activation of the wild-type protein.

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**—Rodney Camire,
The Children's Hospital of
Philadelphia**

In factor VIII knockout mice, expression of the factor IX variant decreased clotting times and blood loss from injury compared with the expression of wild-type factor IX.

Importantly, the factor IX variant retained over half its activity in plasma after 24 hours. The variant did not trigger an immune response in mice that were tolerant to wild-type human factor IX, suggesting the amino acid substitutions were nonimmunogenic.

The results were published in *Blood*.

Schütttrumpf said his group now needs to evaluate its engineered factor IX in hemophilia models using direct protein infusion as opposed to gene transfer.

In addition, both Camire and Schütttrumpf said their groups will evaluate the safety and efficacy of the engineered clotting factors in canine models of hemophilia and carry out additional studies to characterize the molecules' pharmacokinetics.

The German Red Cross Blood Donor Service Baden-Wuerttemberg-Hessen has filed a patent application covering use of the factor IX variant in bleeding disorders. An undisclosed company has licensed the patent, although uses in gene transfer and other areas are still available for licensing.

The Children's Hospital of Philadelphia has issued and pending patents covering factor Xa^{116L} and its use in promoting coagulation.

Pfizer has exclusively licensed the work and has factor Xa^{116L} in preclinical development to treat hemophilia.

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COMPANIES AND INSTITUTIONS MENTIONED

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