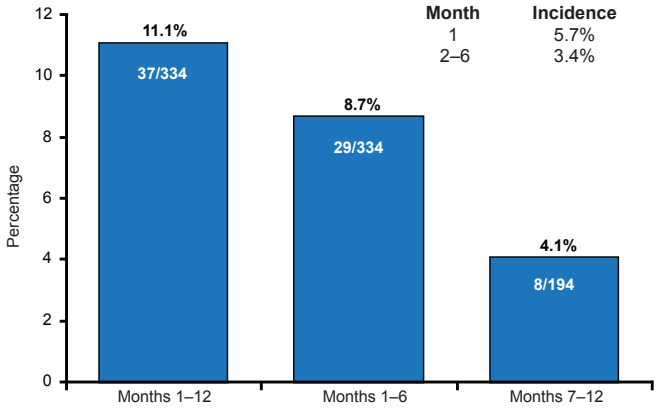
Figure 2. Incidence of New or Recurrent VTE



Extending dalteparin was not associated with an increase in major bleeding and adherence to therapy was high, leading the investigators to conclude that it is feasible to extend dalteparin therapy beyond 6 months in patients with cancer.

Investigational Small Molecule Rapidly Reverses Effects of New Oral Anticoagulants, Heparins

Written by **Wayne Kuznar**

An investigational synthetic antidote for the new oral anticoagulants (NOACs) and heparins demonstrated full reversal of anticoagulation in preclinical studies. The properties and mechanisms of action of the agent, PER977, and preclinical data were discussed by Bryan Laulicht, PhD, Perosphere Inc., Mt. Kisco, New York, USA.

Although new generation Factor Xa and IIa inhibitors offer significant advantages over heparins and warfarin in terms of their route of administration, drug interactions, and predictability of bioactivity, the NOACs lack a specific reversal agent. Thus, concern over the need for rapid reversal should a patient start to bleed or require an emergency procedure when taking an oral factor Xa or IIa inhibitor is heightened.

Dr. Laulicht described PER977, a synthetic small molecule designed as an anticoagulant antidote. PER977 showed no procoagulant effects in human blood on thromboelastography (TEG) *ex vivo*.

In silico modeling data predicted the sites of noncovalent hydrogen bonding between PER977 and new oral anticoagulants and heparins. Data from *in vitro* dynamic light scattering correlate the *in silico*-predicted noncovalent binding specificity of PER977 directly to the approved new generation oral anticoagulants dabigatran, rivaroxaban, apixaban, and edoxaban (approved in Japan only), and heparins.

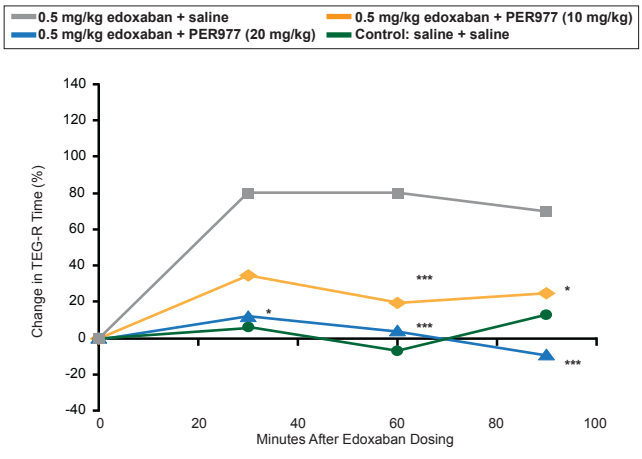
PER977 bonds to 6 sites on heparins, preventing them from binding to antithrombin III. Dynamic light scattering of mixtures of PER977 and enoxaparin provides evidence for the formation of molecular complexes formed at a mass ratio of 1:1, increasing in size at 10:1 ratios, indicating a strong physical, noncovalent association between PER977 and enoxaparin that accounts for the enoxaparin reversal activity of PER977

TEG reaction time (TEG-R) measurements demonstrate a statistically significant decrease ($p<0.01$) back to normal TEG-R levels in enoxaparin-anticoagulated rats within 30 minutes of intravenous administration of PER977 at 30 mg/kg, compared with rats receiving enoxaparin followed by a saline sham.

Preclinical *in vivo* anticoagulant (rat-tail transection bleeding) assays demonstrated improved reversal of enoxaparin with 30 mg/kg of PER977 compared with protamine sulfate ($p<0.05$).

PER977 binds to two sites on edoxaban, preventing it from inhibiting Factor Xa. Within 30 minutes of administration, 10 and 20 mg/kg of PER977 reversed edoxaban anticoagulation in rats measured by TEG in a dose-dependent manner (Figure 1), with full reversal at 20 mg/kg, compared with rats receiving edoxaban followed by a saline sham ($p<0.05$ with 10 mg/kg; $p<0.001$ with 20 mg/kg vs control). Blood loss mass in rats treated with 12.5 mg/kg of oral edoxaban was reduced significantly with administration of PER977 31.25 mg/kg ($p<0.01$).

Figure 1. PER 977 Reverses Edoxaban Anticoagulation in Measured by TEG



*p<0.05; ***p<0.001 With respect to control.

NOTE TO GGI: Interval bars deleted; include "Adapted" in the Permissions line

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PER977 binds to four sites on dabigatran, disallowing it from inhibiting Factor IIa. PER977 31.25 mg/kg significantly reduced blood loss mass in rats treated with dabigatran etexilate 15.5 mg/kg orally ($p<0.001$).

PER977 binds to a site on argatroban that does not interfere with argatroban's binding to Factor IIa. As such, rats administered argatroban 5 mg/kg subcutaneously followed by PER977 100 mg/kg intravenously, remain anticoagulated.

PER977 showed no binding to common cardiac drugs such as lisinopril, propafenone, and digoxin, among others, or to antiepileptic drugs such as gabapentin, lamotrigine, phenytoin, and valproate.

In conclusion, said Dr. Laulicht, PER977 reverses new generation oral anticoagulants *ex vivo* in human blood and decreases bleeding *in vivo* in a standard rat tail bleeding model.

Protein Bioengineering Strategies Improve Upon Hemophilia Treatments

Written by Wayne Kuznar

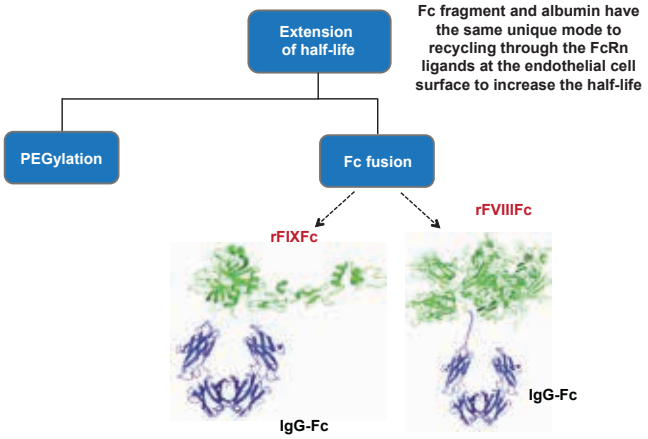
A new era in the treatment of hemophilia has emerged with a new generation of protein therapeutics. Flora Peyvandi, MD, PhD, University of Milan, Milan, Italy, provided an overview of the future of coagulation factor replacement therapy.

Protein engineering has increased the clinical potential and reduced rapid clearance of antihemophilic drugs from the body. Current therapy for hemophilia is safe and effective but immunogenic. Next generation antihemophilic drugs should have enhanced efficacy, greater safety, reduced immunogenicity, and improved delivery. Bioengineering technologies that have been applied successfully to other therapeutic proteins are now being applied to Factor VIII, Factor IX, and Factor VIIa. These technologies include the addition of polyethylene glycol (PEG) polymers and polysialic acids, alternative formulations with PEG-modified liposomes (PEG-Lip), and fusion proteins technologies. Pegylation of a protein extends its half-life and increases drug efficacy.

Glycopegylation allows for targeted pegylation so that the PEG can be attached to specific parts of the coagulation factors, such as the B domain for Factor VIII and the activation peptide for Factor IX. Upon activation, the pegylated portion of the protein is cleaved off, leaving the native activated coagulation factor.

An alternate strategy to extend the half-life of proteins is to fuse them to another protein with a much longer half-life, such as the fragment crystallizable (Fc) region of an immunoglobulin (Figure 1). Fc-containing proteins that are internalized by endothelial cells bind to the neonatal Fc receptor (FcRn) present in the acidified endosome, and are recycled back to the cell surface and subsequently released back into plasma at physiologic pH. These approaches markedly increase molecular weight, which reduces renal clearance.

Figure 1. Half-Life Extension



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Albumin fusion technology yields an altered version of a protein by fusing the gene for human albumin to the gene that encodes the active protein drug. This technology increases the protein's molecular weight, prolonging the half-life *in vivo*. The albumin molecule also masks the protein, rendering it resistant to proteases and less immunogenic.

Modified long-acting recombinant Factor VIII products in late-phase clinical studies have half-lives of 1.5- to 1.6-fold longer than their unmodified versions (Figure 2). Similar strategies have been used to extend the half-life of Factor IX by 3- to 5-fold.

Current prophylactic treatment requires infusion 2 to 3 times weekly using Factor VIII and 2 times weekly using FIX products. In the future, the frequency of administration will be significantly reduced to once or twice weekly and every 1 to 2 weeks with long-acting recombinant FVIII and FIX products respectively, said Prof. Peyvandi.

Bioengineering strategies have also been employed to extend the half-life of recombinant Factor VIIa through site-specific pegylation, albumin fusion, or modification of amino acid sequence.

RNA interference is a cellular pathway of gene silencing in a sequence-specific manner at the mRNA level. A short interfering RNA, ALN-AT3, which employs a hepatocyte-targeting ligand, has been developed against antithrombin. In nonhuman primates, ALN-AT3 yielded potent and