New treatments in hemophilia: insights for the clinician

Karin Knobe and Erik Berntorp

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Abstract: Hemophilia has evolved from an often fatal hereditary bleeding disorder to a disorder for which safe and effective treatment is available. However, there are several challenges remaining in the treatment of hemophilia. Prophylaxis to prevent bleeding is costly and requires frequent intravenous injections, which are cumbersome for patients. Venous access is often difficult to achieve, especially in small children where central venous lines may need to be implanted. Development of inhibitory antibodies makes treatment of acute bleeds difficult and prophylaxis in patients with inhibitors must also be better addressed. In order to improve treatment, new products are being developed, some of which are already in clinical trials. There are several approaches to prolonging half-lives such as PEGylation, Fc fusion and albumin fusion. Increased activity has been demonstrated in preclinical trials for factor IX and in a human trial with factor VII where the activity of the molecules has been increased by manipulation of the molecular composition. Additional approaches, including blockage of inhibitors of clotting, are also under investigation. Factor VIII and factor IX gene therapy have become a tangible possibility since phase I data recently have been published. Results are promising and there is hope that in the near future substantial progress will be made, perhaps making hemophilia the first genetic condition to be cured.

Keywords: coagulation, hemophilia, factor VIII, factor IX, treatment

Introduction

Hemophilia A and B are hereditary X-chromosomal recessive disorders caused by deficiency or absence of coagulation factors VIII (FVIII) or IX (FIX), respectively. The incidence of hemophilia is commonly reported as 1 in 5000 male births or 1 in 10,000 of the general population. Hemophilia A is four times more common than hemophilia B. The disorders are classified according to the coagulation factor activity (FVIII:C or FIX:C, respectively) present in blood with three categories comprising severe (<1% of normal activity), moderate (1–5%) or mild (>5% to <40%) [White *et al.* 2001]. Although these categories define overall bleeding manifestations, the clinical phenotype may vary within each group. Mild hemophilia can go unnoticed depending on the level of deficiency and the stressors the individual experiences, therefore the proportion of cases of mild hemophilia that are registered may vary [Larsson, 1984; Soucie *et al.* 2000]. Severe hemophilia is characterized by spontaneous joint, muscle, gastrointestinal and central

nervous system (CNS) bleeding resulting in substantial morbidity and even mortality. Treatment of hemophilia is based on replacing the missing factor with concentrates containing FVIII or FIX. Mild hemophilia A can usually be treated with desmopressin, thereby increasing the levels of the patient's own FVIII [Mannucci, 2008]. Long-term prophylaxis is becoming widely used in severe forms of hemophilia [Ljung, 2009]. Clotting factors and replacement strategies continue to evolve, mainly for patients without inhibitory antibodies to the missing factor. For patients with inhibitors, treatment for acute bleeding episodes, immune tolerance and, sometimes, immune modulation for inhibitor reduction, are available. In addition, adjuvant therapies such as desmopressin, antifibrinolytics and topical agents contribute to treatment of bleeding in many patients. Cloning of the genes for FVIII and FIX, which occurred over 20 years ago, revolutionized the therapy of both hemophilia A and B. A high proportion of the therapy used today in resource strong countries is recombinant factor

Correspondence to:
Karin Knobe, MD, PhD
Malmö Centre for
Thrombosis and
Haemostasis, Skåne
University Hospital, SE-
205 02 Malmö, Sweden
karin.knobe@med.lu.se
Erik Berntorp, MD, PhD
Malmö Centre for
Thrombosis and
Haemostasis, Lund
University, Skåne
University Hospital,
Malmö, Sweden

concentrates. Initial development of recombinant FVIII was optimized by the co-expression of von Willebrand factor (VWF) that enhances the intracellular trafficking and folding of FVIII. Expression systems that produce FVIII in which the B domain has essentially been removed have been important for production efficiency and also for the early gene therapy attempts since, for most vectors, the size of full-length FVIII is problematic. Recombinant FIX, although clinically effective and with the same half-life as plasma products, has some variability in posttranslational processing which may be the cause of the lower *in vivo* recovery compared with plasma-derived products. Recombinant activated factor VII (rFVIIa) is used to treat patients with hemophilia who have developed inhibitory antibodies. Given the likely addition of new long-acting/long-circulating clotting factors, hemostatic agents that block tissue factor pathway inhibitor (TFPI) and gene therapies, such as gene transfer and premature termination codon suppressors, the future of hemophilia care looks very promising. To understand the forces that drive the development of new treatments in hemophilia, it is important to be knowledgeable about current use of and development of treatments.

Current treatment

Prophylaxis is used in countries that can support the associated medical costs and that give priority to hemophilia care. Prophylactic treatment began in Sweden during the 1950s; a 25-year follow-up of 60 patients was subsequently published in 1992 [Nilsson *et al.* 1992]. The Netherlands also has a long history of use of prophylactic regimens [Van Creveld, 1969, 1971a, 1971b]. The Dutch strategy has differed from the Swedish, primarily with respect to later time of start, lower dosing, and longer dose intervals resulting in less costly regimens [van den Berg *et al.* 2001]. Whereas the Swedish regimen has been relatively fixed, aiming at a trough level of 1% or greater, the Dutch regimen has been tailored to the individual bleeding phenotype.

The trend in Europe is administration of primary prophylaxis [Chambost and Ljung, 2005]. This strategy has been recommended as the standard of care by the Medical and Scientific Advisory Council of the National Hemophilia Foundation, the World Federation of Hemophilia and the World Health Organization [Rodriguez and Hoots, 2010]. It is clear that early start is

important as an independent predictor of future joint disease [Astermark *et al.* 1999; Funk *et al.* 1998; Kreuz *et al.* 1998; Petrini *et al.* 1991]. The challenge with early initiation at the age of 1–2 years remains one of venous access, often requiring the use of a central venous catheter [Ljung, 2002; Petrini *et al.* 2004; Van den Berg *et al.* 2006; Yee *et al.* 2002]. Several authors have recommended beginning therapy with a once-weekly infusion to decrease the need for these devices, and because of the substantial variability of clinical phenotype in severe hemophilia [Aledort *et al.* 1994; Escuriola Ettingshausen *et al.* 2001; Feldman *et al.* 2006; van Dijk *et al.* 2005]. In clinical practice, most prophylactic regimens are tailored to the need of the individual patient. Methods to develop individualized regimens have included use of pharmacokinetic data and computer-simulated dose level and interval to achieve a predetermined trough level [Carlsson *et al.* 1993, 1998]. Decreasing intervals between prophylactic doses from 2–3 infusions weekly to every other day theoretically reduces average FVIII consumption by 43% with maintained or increased trough FVIII levels, while daily dosing would reduce mean FVIII usage by 82%. A modified dosage regimen with infusions every other day was implemented in a group of Swedish patients with data obtained supporting the pharmacokinetic models [Carlsson *et al.* 1993]. Patients who have experienced joint bleeds may, in addition to the treatment of bleeds, be treated with periodic use of factor concentrates. This approach is known as secondary prophylaxis and is commonly used to minimize bleeding frequency and minimize the progression of joint disease. Secondary prophylaxis cannot reverse, but can decrease the progression of chronic arthropathy. It reduces the frequency of bleeding, hospital admissions and lost days from school or work. The use of secondary prophylaxis *versus* on-demand therapy has been studied in children and adults, the results indicating that secondary prophylaxis results in a decreased number of joint bleedings [Aledort *et al.* 1994; Smith *et al.* 1996; Szucs *et al.* 1998]

Inhibitors develop in approximately 30% of patients with severe hemophilia A and are less common, ~5%, in those with severe hemophilia B [Astermark, 2006]; a broad range of inhibitor incidence/prevalence has been reported among different studies. A variety of factors may impact inhibitor development including but not limited to: type of hemophilia, level of deficiency, specific

genetic mutation, race, immune response genes, and environmental influences. Among environmental issues suggested are the type of clotting factor therapy utilized, such as plasma derived *versus* recombinant, intermediate *versus* high-purity products, treatment regimen (prophylaxis *versus* on-demand), peak treatments and age at start of treatment, etc. [Calvez and Laurian, 2006; Gouw *et al.* 2007b; Santagostino *et al.* 2005].

During recent years, there has been discussion as to whether recombinant products are more immunogenic than plasma-derived intermediate purity products, especially those with intact von Willebrand factor (VWF) [Goudemand *et al.* 2006; Gouw *et al.* 2007a; Iorio *et al.* 2010; Scharrer *et al.* 1999]. Preclinical research addressing of if and how vWF or other proteins regulate the immune response have been published [Dasgupta *et al.* 2007; Qadura *et al.* 2009]. Despite active debate regarding this issue, the currently available support for either position is of relatively low scientific value: no prospective randomized study has been performed to address the issue. A recent European consensus report stated that there is no difference in risk of inhibitor development between different concentrate types [Astermark *et al.* 2006]. The question regarding type of concentrate and rate of inhibitor development are being addressed by collection of registry data such as the EUHASS registry [Makris *et al.* 2011] and by the SIPPET study, where classes of products are being compared in a randomized prospective fashion [Mannucci *et al.* 2007].

The most important issues with respect to hemophilia treatment are use of prophylaxis, and inhibitor development:

- Treatment with factor concentrates requires intravenous injections, a burden that negatively impacts quality of life. As prophylaxis should be started early [Astermark *et al.* 1999] small children are in need of ports to provide venous access, requiring a surgical procedure early in life with its general risks, and, in addition, surgical procedures may be a risk factor for inhibitor development [Gouw *et al.* 2007b]. Ports are a source for septic infections [Ljung *et al.* 1992]. Novel products with longer biological half-life have a potential to increase convenience and reduce the need for port implantations. Such products are now in clinical trials.

- Inhibitor risk is associated with genetic factors [Astermark, 2006] especially the disease causative mutation [Oldenburg *et al.* 2002]. This means that treatment with FVIII in a patient with a high-risk mutation has a substantial risk for development of an inhibitor. Methods for avoidance of inhibitors remain an important, but unmet, need. There are clinical trials attempting to address this with currently available products. One, for example, controls the treatment regimen by early start of prophylaxis in relatively low doses [ClinicalTrials.gov identifier: NCT01376700]. A logical rationale would be to treat individuals at high risk with products not recognized as FVIII by the immune system. Such products are in early development. In summary, the possibility to predict development of an inhibitor, and learn to avoid this, remain to be elucidated.
- Patients having high-titer inhibitors cannot be treated using FVIII replacement therapy. Therefore, bypassing products have been used for many years. The currently available products are rFVIIa and activated prothrombin complex concentrate (aPCC). These drugs are very different in terms of content and mode of action, but seem to have rather similar overall effectiveness with regard to treatment of acute bleeds [Astermark *et al.* 2007] with efficacy rates of the order of 80–90% [Astermark *et al.* 2006], leaving a proportion of patients without effective treatment. Obviously, there is a need for products that can promote and predict hemostasis in a secure way when an inhibitor is present. New products for this patient group are under development, and molecules with increased activity are in clinical trials. Prophylactic therapy for this patient group would be a step forward; studies are being undertaken for this purpose with new molecules.

New products and approaches to treatment

New, improved products or treatment approaches for hemophilia A or B would significantly improve the lives of patients. Current therapy is cumbersome and intermittent, due to the need of intravenous injections, with peaks and troughs in plasma factor levels allowing for breakthrough bleeding episodes. Both FVIII and FIX have

Table 1. Currently ongoing product development.

Technique used	Product	Published clinical results hemophilia indication	Clinical studies	ClinicalTrials.gov identifier
PEGylation	FVIII FIX FVIIa	No Yes [Negrier <i>et al.</i> 2011] Yes [Moss <i>et al.</i> 2011]	2 Phase I/II compl., Phase III open Phase I/II compl., Phase III open Phase I compl., Phase II compl.	NCT01184820, NCT01205724, NCT01480180 NCT00956345, NCT01333111 NCT00922792, NCT00951405
PEGylated liposomes	FVIII	Yes [Spira <i>et al.</i> 2006, 2008, 2010]	Phase III and project closed due to lack of efficacy	NCT00245297, NCT00629837, NCT00623727
Polysialic acid	FVIII	No	–	–
Albumin fusion	FVIIa FIX	No No	– Phase I ongoing	– NCT01361126, NCT01233440
Fc fusion	FVIII FIX	No Yes [Shapiro <i>et al.</i> 2011]	Phase I/II compl., Phase III compl. Phase I/II compl., Phase III compl.	NCT01027377 NCT00716716
Increased activity	FVIIa	Yes [Moss <i>et al.</i> 2009]	Phase I and II compl. Phase III ongoing	NCT00486278, NCT01392547
Gene therapy	FVIII FIX	Yes, reviewed by Pierce <i>et al.</i> [2007]	Two phase I trials ongoing	NCT00979238, NCT00515710
Anti-TFPI	antibody aptamer	No No	Phase I ongoing Phase I registered	NCT01228669 NCT01191372
Read-through of premature stop codons	Hemophilia A or B	No	Phase II study suspended	NCT00947193
Transgenic animals	FVIII FIX	No	–	–
Transgenic tobacco plants	FIX	No	–	–

TFPI, tissue factor pathway inhibitor

wide therapeutic windows and slight increases in factor levels will reduce morbidity. Treatment that provides more ‘close to normal’ levels would allow a lifestyle similar to people without a bleeding disorder. The most well-known techniques for new products are described and summarized in Table 1. Information provided has been obtained from ClinicalTrials.gov.

PEGylation

PEGylation is an established method for prolonging the half-life of protein products and it has successfully been used in several therapeutic proteins [Fried *et al.* 2002; Keating and Curran, 2003]. The volume of PEGylated proteins is increased, leading to reduced renal clearance and shielding effects may diminish receptor mediated clearance, susceptibility to proteolytic degradation

and reduction in immunogenicity [Webster *et al.* 2007]. For FVIII but maybe not for FIX, PEGylation is likely to have more limited advantage compared with other biologics, as the FVIII molecule is large enough not to be filtered by the kidney. Instead, PEGylation may reduce interaction with clearance receptors in the liver. Another benefit could be reduction of contact with inactivating proteases and immune-mediating cells. Variants have been engineered with a polyethylene glycol (PEG) polymer specifically conjugated to parts of the molecule and preclinical data are published from two groups using different approaches [Holmberg *et al.* 2009; Mei *et al.* 2010; Ostergaard *et al.* 2011]. In bleeding models of hemophilic mice, PEGylated FVIII not only exhibited prolonged efficacy that is consistent with the improved pharmacokinetics but also showed efficacy in stopping acute bleeds

comparable with that of unmodified rFVIII [Mei *et al.* 2010]. The same has been shown for PEGylated FIX [Holmberg *et al.* 2009; Ostergaard *et al.* 2011]. Clinical trials with PEGylated FVIII and FIX have been initiated. So far, data from one FIX phase I/II trial have been published showing a half-life of 93 hours, five times greater than previous products [Negrier *et al.* 2011], and a phase III trial is ongoing [ClinicalTrials.gov identifier: NCT01333111]. Two phase I/II trials with PEGylated FVIII, from different companies, have been completed and one phase III trial initiated [ClinicalTrials.gov identifier: NCT01480180]. PEGylated FVIIa has also been developed to meet the need of prophylactic therapy for inhibitor patients. A phase I trial has been published [Moss *et al.* 2011] and a phase II trial in patients has been completed [ClinicalTrials.gov identifier: NCT00951405]. In the case of FVIIa the PEG is attached to the active molecule and not attached to the zymogen and cleaved off when activated as done for FVIII and FIX

The use of *PEGylated liposomes* has also been tried in humans [Spira *et al.* 2010].

Liposomes can be efficacious vehicles for medicines, and surface modification by PEGylation can prolong liposome circulation time. Use of this preparation was associated with increased protection from bleeding [Spira *et al.* 2006]. The product (KG-Lip) was further evaluated in a large clinical trial, the Liplong study, where once-a-week treatment with the product was compared with Kogenate FS Bayer. The study was stopped because the KG-Lip product failed to meet the endpoints and was inferior to the comparator product which was given three times per week.

Fc fusion

Fc fusion, the neonatal Fc receptor (FcRn), is a MHC class I like molecule that functions to protect IgG and albumin from catabolism, mediates transport of IgG across epithelial cells, and is involved in antigen presentation by professional antigen presenting cells. The ability of this receptor to prolong the half-life of IgG and albumin has guided development of novel therapeutics [Kuo *et al.* 2010]. Peters and colleagues have summarized studies in which Fc has been fused to FIX [Peters *et al.* 2010]. Taken together, these studies showed the enhanced pharmacodynamic and pharmacokinetic properties of the rFIXFc fusion protein and provided the basis for evaluating

rFIXFc in patients with hemophilia B. A recombinant fusion protein (rFIXFc) containing a single FIX molecule attached to the Fc region of immunoglobulin G was administered intravenously and found to have an extended half-life, compared with recombinant FIX (rFIX) in hemophilia patients. The phase I trial has been completed for rFIXFc [ClinicalTrials.gov identifier: NCT00716716] and published results showed an approximate threefold increased half-life compared with current products [Shapiro *et al.* 2011]. Recruitment in the FVIII phase I trial is also completed [ClinicalTrials.gov identifier: NCT01027377]. Clinical trials (phase II/III) in patients with hemophilia A and B are fully recruited or completed and results will soon be published.

Albumin fusion

Albumin has a long half-life, exceeding 20 hours. As albumin is a product with a strong safety record, and does not appear to be immunogenic, it presents an option for extension of clotting factor half-life using genetic fusion. Weimer and colleagues reported a recombinant FVIIa molecule with an extended half-life based on genetic fusion to human albumin [Weimer *et al.* 2008]. The recombinant FVII albumin fusion protein (rVII-FP) was expressed in mammalian cells and upon activation displayed a FVII activity close to that of wild type FVIIa. Pharmacokinetic studies in rats demonstrated that the half-life of the activated recombinant FVII albumin fusion protein (rVIIa-FP) was extended compared with wild-type rFVIIa. Albumin has also been fused to FIX and two phase I/II trials are currently registered [ClinicalTrials.gov identifiers: NCT01361126 and NCT01233440]. Results are not yet available.

Increased activity of molecules

It has been possible to increase the catalytic activity of FIX using different techniques: replacing the epidermal growth factor (EGF)-like domain of FIX with that of FVII [Chang *et al.* 1997] or changing residue 338 in human FIX from arginine to alanine [Chang *et al.* 1998]. Intensifying the catalytic activity has an obvious potential to construct a drug which is more efficacious for treatment of hemophilia B, but to date the concept has not been tested in human trials. For FVII the progress has come further, although much information is available only in abstract form, and clinical trials are ongoing or planned. Novo Nordisk A/S as well as Bayer Healthcare have

developed FVIIa molecules with increased activity compared with the currently available product (NovoSeven®, Novo Nordisk A/S). The rFVIIa analog (NN1731, Novo Nordisk A/S) has three amino-acid substitutions that stabilize the molecule in its active conformation in the absence of tissue factor. Preclinical data indicate that compared with rFVIIa the analog has a more rapid onset of action, forms a stronger clot that is more resistant to fibrinolysis and exhibits an improved therapeutic window [Allen *et al.* 2007; Holmberg *et al.* 2009; Sorensen *et al.* 2007]. The product has been administered to healthy subjects [Moss *et al.* 2009], a phase II trial in patients has been completed [ClinicalTrials.gov identifier: NCT00486278] and the phase III trial is recruiting [ClinicalTrials.gov identifier: NCT01392547]. Results of clinical trials must be completed before the future of these products can be evaluated. There is a need for more efficacious products to treat bleeds in inhibitor patients and perhaps also for prophylaxis. Safety aspects are important and the potential increased risk of thromboembolism needs to be carefully evaluated.

Polysialic acid

Polysialic acid (PSA) is an anionic moiety that adds multiple negative charges to the protein thereby changing its surface charge and binding capabilities [Pisal *et al.* 2010]. PSA is thought to be able to interfere with receptor-mediated clearance processes of FVIII. The compound is under preclinical evaluation, and no data available at this time.

Gene therapy

Hemophilia is an attractive disease for gene therapy for many reasons. It is a relatively prevalent disorder compared with many monogenetic disorders and the functions of the missing proteins are well studied. Both FVIII and FIX have wide therapeutic windows and slight increases in factor levels will reduce morbidity. Endogenous FVIII and FIX are secreted by endothelial cells and hepatocytes but can be expressed in other types of cells, thereby increasing the options for gene transfer. Five gene transfer phase I clinical trials have been conducted using either direct *in vivo* gene delivery with viral vectors or *ex vivo* plasmid transfections and reimplantation of gene-engineered cells [Pierce *et al.* 2007]. Although there was evidence of gene transfer and therapeutic effects in some of these trials,

stable expression of therapeutic FVIII or FIX levels has not yet been obtained. Further improvements of the vectors and a better understanding of the immune consequences of gene transfer are warranted and ongoing. There are two phase I/II gene therapy trials in hemophilia B registered [ClinicalTrials.gov identifiers: NCT00979238 and NCT00515710]. The publication of a successful gene therapy approach in adenosine deaminase-deficient severe combined immunodeficiency (SCID) patients [Aiuti *et al.* 2009] and the recent publication of gene transfer in hemophilia B [Nathwani *et al.* 2011] provides hope that gene therapy is a possibility.

Blockade of tissue factor pathway inhibitor

Blockade of an inhibitor of clotting, tissue factor pathway inhibitor (TFPI) is another interesting approach. A class of therapeutics that is being investigated is the nonanticoagulant sulphated polysaccharides (NASP). They are believed to act through blockade of TFPI. NASP can accelerate the clotting times of plasma from hemophilia patients, improve hemostasis when administered subcutaneously to hemophilic mice [Liu *et al.* 2006] and improve hemostasis when administered orally to severe hemophilia A dogs [Prasad *et al.* 2008]. A new technology, aptamers, which are single-stranded nucleic acids, can also be used to block TFPI. Aptamers directly inhibit function by folding into a specific structure with high affinity for the target [White *et al.* 2000]. This technology is being explored in clinical trials of other disorders and a trial with an anti-TFPI-aptamer is registered although not yet recruiting [ClinicalTrials.gov identifier: NCT01191372]. An anti-TFPI antibody is in a phase I clinical trial [ClinicalTrials.gov identifier: NCT01228669]; no data are available as of yet.

Other approaches

Some of the mutations causing hemophilia are nonsense changes. Small molecules have been developed that can read-through premature stop codons. Ataluren (PTC124) is an orally delivered, investigational drug that acts to overcome the effects of the premature stop codon [Hirawat *et al.* 2007; Welch *et al.* 2007], potentially enabling the production of functional FVIII/FIX [ClinicalTrials.gov identifier: NCT00947193]. The study was suspended, no reason was given. Another approach is to develop pharmaceuticals using transgenic animals and plants. Both FVIII

and FIX have been produced in transgenic pigs as bioreactors [Paleyanda *et al.* 1997; Pipe *et al.* 2011; Van Cott *et al.* 1999] and FIX in chloroplast transgenic tobacco plants [Verma *et al.* 2010]

Concluding remarks

The advantage of long-acting products or gene therapy is obvious. A reduction of intravenous punctures is especially important for pediatric patients. Less frequent need for venous access means fewer placements of indwelling catheters. Furthermore, frequent dosing interrupts daily activities and many patients must rely on help from family or sometimes even hospital personnel. Compliance with frequent injection regimens is one of the most commonly cited reasons for failure of prophylaxis [Geraghty *et al.* 2006]. In addition, a single dose may be sufficient for intensive treatment of a bleeding episode, which would be an advantage especially for patients that must travel for help with injections. A concern related to the half-life of the product and how seldom it is dosed, is that the factor levels in plasma, although measurable, could be rather low during a substantial number of hours. Given the broad variation in clinical phenotype, some patients are at risk of bleeding at FVIII/FIX levels which exceed those that may be obtained with some products during a substantial portion of the week [Ahnstrom *et al.* 2004]. This fear is even more relevant if the patient is physically active, involved in sports, etc. Therefore, extra doses of concentrate may be needed prior to activity. This could be an extra dose with a product with shorter half-life: an approach similar to that used for insulin treatment. However, this approach for hemophilia can only be considered second best since, if possible, at an acceptable cost, there is no known disadvantage to continuously have 50–80% of normal FVIII or FIX activity levels.

The advent of more potent bypassing products opens up the possibility of treating acute bleeds better and more reliably. A more potent and perhaps longer-acting bypassing agent could open the possibilities for efficacious prophylaxis for patients with inhibitors. Anti-TFPI, which perhaps could be administered subcutaneously or orally, is another possibility for this patient group. As inhibitors develop early in life, institution of effective prophylaxis could be a reality before the onset of joint disease, as is the case in children without inhibitors, i.e. as primary prophylaxis.

Development of an inhibitor is a major concern and threat to the health of the person with hemophilia. If a reliable mechanism, such as a score for predicting inhibitor risk could be developed, new products or new treatment regimens could hypothetically be used to avoid challenges to the immune system in such patients. An example would be to give biosuperior FVIIa or anti-TFPI to patients with hemophilia A and a very high risk score for developing an inhibitor. So far such attempts however do not seem to have been successful [Rivard *et al.* 2005]. The future will tell us whether such approaches will become reality.

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Conflict of interest statement

Erik Berntorp has received speakers fees and is on the Advisory Boards of Baxter BioScience, Novo Nordisk, Bayer, Pfizer, CSL Behring, Biogen-Idec Pharmaceuticals and Octapharma. Karin Knobe has previously been employed by Novo Nordisk.

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