The Hemophilia Report

Selected Reports from the
65th Annual Meeting of the National Hemophilia Foundation and
55th Annual Meeting of the American Society of Hematology

Steven W. Pipe, MD
Guest Editor

Differentiating Hemophilia A and B  Genetics of Hemophilia
Prophylactic vs Episodic Therapy  Advances in Clotting Factors
Avoiding Inhibitors  Managing Acute Bleeding
Novel Therapeutics  What’s in the Pipeline?

CONTINUING EDUCATION FOR PHYSICIANS AND NURSES: 3.5 CREDITS AVAILABLE

This activity is supported by an educational grant from Biogen Idec.
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Steven W. Pipe, MD, University of Michigan, Ann Arbor, Michigan

Meeting the Challenges of Managing Hemophilia: Prophylactic vs Episodic Therapy and Avoiding Inhibitors
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Anna Chalmers, MD, Rush University Medical Center, Chicago, Illinois

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Recent Advances in Preventing Bleeding, Reducing Inhibitors, and Managing Acute Bleeding
Noa Biran, MD, Tisch Cancer Institute, Mount Sinai School of Medicine, New York, New York

CME/CNE Post Test and Evaluation
About This CME/CNE Activity

RATIONALE AND PURPOSE
Hemophilia, an X-linked recessive bleeding disorder, affects more than 400,000 people worldwide. Internal bleeding related to the disorder spans the gamut from spontaneous hemarthroses resulting in chronic, disabling joint pain and swelling to deep-muscle, central nervous system, and gastrointestinal bleeding. This inaugural issue of The Hemophilia Report explores the genetic foundation of hemophilia, differentiates between its two major types (A and B), and reviews the current management of this hematologic disease, as well as recent advances in preventing bleeding and reducing the development of autoimmune inhibitors to coagulation replacement factor therapy.

Among the many topics discussed by the authors are advances in synthetic blood-coagulation factors that promise to improve patient outcomes with more effective bleeding control and preservation of joint function; reducing the burden of prophylactic replacement therapy by extending the half-life of bioengineered coagulation factors; personalizing treatment based on the pharmacokinetics of replacement factors, bleeding phenotypes, and lifestyle; identifying, monitoring, and preventing age-related comorbidities; and potentially developing a cure for hemophilia through gene therapy. The articles in this issue are based upon presentations delivered during the 65th Annual Meeting of the National Hemophilia Foundation, held in Anaheim, California, October 3–5, 2013, and the 55th Annual Meeting of the American Society of Hematology, convened in New Orleans, Louisiana, December 7–10, 2013.

The articles in this issue, written from the academic perspective of physicians-in-training at leading medical institutions, summarize the import of these new findings and place them into clinical context. This activity has been developed and approved by a planning committee of nationally recognized thought leaders to meet a perceived educational need to provide hematologists, other physicians, and nurses with diagnostic and therapeutic strategies to help them perform their clinical roles.

LEARNING OBJECTIVES
After studying this issue of The Hemophilia Report, participants in this educational activity should be able to:

• Outline the genetic basis of hemophilia A and B and differentiate between the two types.

• Review the history of hemophilia management, discuss current best practices in children and adults, and explain the goals of ongoing research to better understand the disease.

• Summarize the developments being made in the laboratory and currently being tested in clinical trials to produce synthetic coagulation factors with longer half-lives than those of present blood products for treating hemophilia or that are less prone to stimulate the production of neutralizing antibodies (inhibitors).

• Discuss the advantages and drawbacks of prophylactic versus on-demand therapy, the controversies over therapeutic timing and individualizing therapy for different patients, the factors affecting patient compliance, and the promise of bioengineering and pharmacokinetic strategies to individualize patient management.

TARGET AUDIENCE
Hematologists, other physicians, and nurses significantly involved in the management of hemophilia should find participating in this educational activity valuable.

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This Enduring Material Activity is available in print and online at www.HemophiliaReport.com and consists of an introduction, seven articles, a postactivity assessment, and an evaluation. Estimated time to complete the activity is 3.5 hours.

To receive credit, participants must read the CME information on these two pages, including the learning objectives and disclosure statements, as well as the full content of this monograph, and then complete the post test and evaluation.
form online at www.HemophiliaReport.com. Upon successful completion of the post test (80% correct) and evaluation form, a CME certificate of participation will be awarded automatically. The certificate may be printed directly from the Web site or e-mailed and printed later.

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Jacqueline Keenan and Edwin Geffner of Direct One Communications, Inc., have nothing to disclose.

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In this issue of The Hemophilia Report, Dr. Hege discusses the rationale for development of new, longer-acting coagulation replacement factors and what’s in the pipeline. Dr. Biran describes a recombinant factor VIII crystallizable fragment (Fc) fusion protein currently under FDA review and the off-label use of a four-factor prothrombin complex concentrate (PCC) in patients requiring anticoagulant reversal before undergoing urgent surgical procedures. Dr. Chalmers refers to the potential of PEGylated and fusion protein replacement factors and gene therapy. Drs. Husseinzadeh and Sung discuss recent clinical trials of these and other investigational replacement factors, as well as novel bypassing agents, PCCs, and gene-transfer therapy. Dr. Tran also touches on the potential of gene therapy.

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About This CME/CNE Activity
Introduction

Selected Reports from the 65th Annual Meeting of the National Hemophilia Foundation and 55th Annual Meeting of the American Society of Hematology

Steven W. Pipe, MD, Guest Editor
University of Michigan, Ann Arbor, Michigan

Exploring hemophilia and its ramifications involves a myriad of medical disciplines—hematology, immunology, genetics, infectious disease, radiology, nursing, and pharmacology, to name a few. During two recent professional gatherings of hematologists and other health professionals, an international cadre of experts on hemophilia reviewed our current understanding of this difficult disease and shared insights on its management and impact on society.

Some 3,000 healthcare professionals attended or participated in over 40 educational sessions offered during the 65th Annual Meeting of the National Hemophilia Foundation, held in Anaheim, California, October 3–5, 2013, to better understand this disease and associated issues. During the 55th Annual Meeting of the American Society of Hematology, which took place in New Orleans, Louisiana, December 7–10, 2013, over 18,000 hematologists and other healthcare professionals took part in scientific sessions and presentations, many of which involved the etiology, diagnosis, and treatment of bleeding disorders. This premiere edition of The Hemophilia Report delves into subjects pertinent to the practices of hematologists and other healthcare professionals involved in managing hemophilia.

HEMOPHILIA THERAPY

Duc Q. Tran, MD, from Emory University in Atlanta, Georgia, provides a historic perspective on the treatment of hemophilia. Among the subjects he covers are the successes and challenges of prophylactic replacement factor therapy in children; in particular, the results of the Joint Outcome Study and the Evaluation Study on Prophylaxis: a Randomized Italian Trial (ESPRIT) are compared and contrasted with data collected during other clinical trials of prophylactic regimens. Dr. Tran also reviews the major studies on prophylaxis versus episodic, on-demand therapy in adults and the current options for managing the development of alloimmune inhibitory antibodies to factor VIII (FVIII).

Anthony Sung, MD, from Duke University Medical Center in Durham, North Carolina, summarizes the history of hemophilia therapy and a number of discoveries that are advancing treatment of the disease. He writes about strategies toward longer-acting clotting factors through the use of PEGylation, the attachment of polyethylene glycol (PEG) molecules to recombinant factor proteins, and fusion protein technologies, among others, that promise to improve the efficacy of treatment and reduce the burden of infusions. The latest safety and efficacy data from clinical trials with these novel agents are provided. Dr. Sung then reviews challenges to reversal and inhibition of bleeding presented by the use of older and newer oral anticoagulants. The efficacy of prothrombin complex concentrates and recombinant activated factor VII for traumatic or surgical bleeding and reversal of bleeding complications resulting from oral anticoagulation are detailed.

CHALLENGING DECISIONS

Anne Chalmers, MD, from Rush University Medical Center in Chicago, Illinois, compares hemophilia A with hemophilia B and confronts challenging treatment decisions. When treatment should be started, what the optimal dose of certain therapeutic agents should be, and how long prophylactic therapy should continue remain controversial. A historic view of hemophilia management has led to greater regard for prophylaxis rather than symptomatic treatment, even though optimal prophylactic regimens and schedules continue to be explored.

In a separate article, Dr. Chalmers reviews our current understanding of the genetics of hemophilia A and B. She addresses the spectrum of genetic mutations that lead to development of each disease, strategies for genetic testing, and how data from such tests could be used to guide treatment.

NOVEL THERAPEUTICS

Holleh D. Husseinzadeh, MD, from the Hospital of the University of Pennsylvania in Philadelphia, covers recent advances in novel therapeutics for bleeding disorders,
including gene-transfer options. Also discussed are new insights into the application of prophylaxis for hemophilia complicated by inhibitors; issues that affect compliance with therapy; and outcomes of total joint replacement in patients with congenital bleeding disorders.

MEETING THE CHALLENGES AHEAD

Kerry Hege, MD, from the Indiana University School of Medicine in Indianapolis, takes a closer look at the improved outcomes and remaining challenges with prophylactic therapies for hemophilia and the results of clinical trials with current state-of-the-art agents. Dr. Hege describes efforts to increase patient adherence to prophylactic regimens by prolonging the half-life of recombinant factor products (and thereby reducing the need for frequent dosing), prevent bleeding episodes, and improve patient quality of life. Among the exciting subjects she covers are PEGylation, polysialylation, fusion protein technology, and gene therapy.

In recent years, many advances in preventing bleeding, reducing the risk of inhibitor formation, and managing acute bleeds in hemophilia have been reported. Noa Biran, MD, from the Mount Sinai School of Medicine in New York, reports on studies investigating the use of recombinant FVIII and factor IX crystallizable fragment fusion protein in patients with severe hemophilia A or B, respectively. Dr. Biran also reviews the results of studies comparing early prophylactic measures with episodic therapy, noting that prophylaxis has resulted in superior results. He also reports on the usefulness of magnetic resonance imaging to monitor hemophilic joint disease, covers strategies to reduce the incidence of inhibitors, and touches upon methods to limit acute bleeding.

By sharing the information imparted by hemophilia experts at these two important medical meetings, the authors of this report greatly enhance our understanding of many complicated issues involved in hemophilia prophylaxis and management. Future editions of The Hemophilia Report certainly will add to these insights and further assist busy practitioners and nurses in keeping abreast of novel developments in the optimal management of hemophilia.
Meeting the Challenges of Managing Hemophilia: Prophylactic vs Episodic Therapy and Avoiding Inhibitors

Duc Q. Tran, MD
Winship Cancer Institute, Emory University, Atlanta, Georgia

Abstract Management of hemophilia has changed over the past few decades. However, the most common complication of hemophilia remains the development of joint bleeds leading to hemophilic arthropathy. Coagulation factor replacement for children should be started early and given prophylactically to preserve joint health and prevent complications. For adults started on prophylaxis, limited studies have reported decreased bleeding episodes, but improvement of joint function and quality of life remains unclear. Inhibitor formation as a complication of coagulation factor replacement and its management are reviewed. Future potential therapies for patients with hemophilia are summarized briefly.

Hemophilia is an X-linked recessive bleeding disorder that results from deficiencies in coagulation factors VIII (FVIII) and IX (FIX). Decreased or absent levels of these factors lead to hemophilia A and B, respectively. The severity of hemophilia is classified as mild, moderate, or severe depending on factor activity levels. Mild disease is associated with a factor activity level > 5 IU/dL and < 40 IU/dL; moderate disease with a factor activity level of 1–5 IU/dL; and severe disease with a factor activity level of < 1 IU/dL.

The prevalence of hemophilia A is 1:5,000 male live births and of hemophilia B, 1:30,000 male live births. The most common morbidity noted among patients with severe disease is spontaneous joint bleeding (hemarthrosis), which can affect any joint but which primarily affects the ankles, knees, and elbows. In addition to spontaneous hemarthrosis, patients with severe disease also can develop spontaneous soft-tissue, gastrointestinal, and central nervous system bleeding. Recurrent hemarthroses can lead to debilitating joint disease, also known as hemophilic arthropathy. Patients with moderate disease usually bleed in response to some injury but can have spontaneous bleeding; patients with mild disease typically only bleed in response to major insults such as surgery or significant trauma.

PROPHYLAXIS IN CHILDREN

The cornerstone of hemophilia management is coagulation factor replacement. Over the past two decades, the focus of therapy has shifted from treatment after bleeding has occurred to prevention of bleeding to allow patients to have a more active and fuller lifestyle. Prophylactic factor replacement was first pioneered in Sweden in 1958, when patients with moderate hemophilia were found to be less likely to have chronic debilitating joint disease than patients with severe hemophilia.

Two recent, randomized, controlled trials that compared prophylaxis with episodic treatment showed definitive benefits with early prophylaxis. Results of these studies—the Joint Outcome Study in the United States and the Evaluation Study on Prophylaxis: a Randomized Italian Trial (ESPRIT)—are summarized in Table 1. Both evaluated the use of prophylactic factor replacement in young boys who had either no or few joint bleeds.

Joint Outcome Study

The Joint Outcome Study enrolled 65 male children with a history of minimal or no joint bleeding (Table 1). The subjects, who were < 2.5 years old at study entry, were randomized to receive either prophylactic or episodic treatment. Children in the prophylactic treatment arm received 25 IU/kg of recombinant FVIII (rFVIII) every other day; those in the episodic treatment arm received 40 IU/kg of rFVIII during each bleed, followed by a single dose of 20 IU/kg 24 and 72 hours later.

As shown in Figure 1, the frequency of bleeding was significantly less in the prophylactic treatment arm than in the episodic treatment arm. As the children grew older and more active, those in the...
ESPRIT

The ESPRIT investigators evaluated 40 older male children (median age, 48–50 months) at study entry and who had no physical or radiographic arthropathy (Table 1). These children were randomized to receive either prophylactic or episodic treatment. Boys in the prophylactic treatment arm received 25 IU/kg of rFVIII three times/week, with doses up to 40 IU/kg to obtain trough levels > 1%. Those in the episodic treatment arm received ≥ 25 IU/kg of rFVIII during each bleed, with repeated doses every 12–24 hours until the bleeding resolved. As shown in Table 3, children in the prophylactic treatment arm experienced fewer bleeding episodes and had less radiographic evidence of arthropathy than those in the episodic treatment arm.5 Quality-of-life (QOL) measures differed significantly between the two groups because the children in the episodic treatment arm had a greater sense of overprotection.5

In both studies, significantly more replacement factor was used in the prophylactic treatment arm than in the episodic treatment arm. In addition to greater cost due to more factor consumption, there was also a higher rate of complications related to the placement of indwelling catheters to maintain the prescribed prophylactic treatment regimen.

Other Studies in Children

Other prophylactic approaches also have been used. In the Canadian tailored prophylaxis trial conducted by Feldman et al.,25 boys began primary prophylaxis with 50 IU/kg of rFVIII once weekly, followed by stepwise increases to 30 IU/kg twice weekly and then 25 IU/kg every other day, depending on the frequency of bleeding episodes. After 5 years, 10 of the 25 children were receiving weekly infusions, 8 were being treated twice weekly, and 7 were being treated every other day. At the end of the study, all of the children had normal joints by physical examination or x-ray.5

Mean number of hemorrhages per month

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Joint Outcome Study (JOS)</th>
<th>ESPRIT Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean)</td>
<td>&lt; 2.5 (1.6)</td>
<td>1–7 (4.1)</td>
</tr>
<tr>
<td>Prior bleeding</td>
<td>≤ 2 bleeds in index joints (prophylactic therapy arm: 1; episodic treatment arm, 0.6)</td>
<td>≤ 2 bleeds in a single site (unknown)</td>
</tr>
<tr>
<td>Baseline arthropathy</td>
<td>None by physical or x-ray examination</td>
<td>None by physical or x-ray examination</td>
</tr>
<tr>
<td>Treatment regimen (recombinant factor VIII)</td>
<td>Prophylactic therapy arm: 25 IU/kg every other day</td>
<td>Prophylactic therapy arm: 25 IU/kg 3 times/wk, up to 40 IU/kg per dose</td>
</tr>
<tr>
<td>Episodic treatment arm:</td>
<td>40 IU/kg as needed, then 20 IU/kg at 24 and 72 hours</td>
<td>Episodic treatment arm: ≥ 25 IU/kg as needed, then every 12–24 h as needed</td>
</tr>
</tbody>
</table>

FIGURE 1 Frequency of bleeding events in the Joint Outcome Study among boys receiving prophylactic or episodic factor replacement therapy. Adapted, with permission, from Manco-Johnson et al.8

as compared with the cost of a standard high-dose prophylactic regimen over that 5-year period.7

In the Netherlands, Fischer and colleagues4 studied a different approach. Instead of using the standard high-dose prophylactic regimen, they started prophylaxis with lower, intermediate doses (usually 15–25 IU/kg 2–3 times/wk) when joint bleeding began. When compared with a Swedish (high-dose) cohort, the Dutch (intermediate-dose) cohort used less than half as many units of FVIII per patient per year.8 Outcomes in the Dutch cohort were good but somewhat inferior to those in the Swedish cohort, since some increase in bleeding and more clinical evi-
Hemophilic arthropathy and improves FIX activity levels ≥ 1% in children with hemophilia A. Because comparative data on patients with hemophilia B receiving prophylactic versus episodic treatment are limited, most treatment centers extrapolate the results from patients with hemophilia A and apply similar principles to maintain FIX activity levels ≥ 1% in children with hemophilia B.

PROPHYLAXIS IN ADULTS

About one third of young adults who received primary prophylaxis as children choose to switch to episodic treatment. One analysis of a self-reported cohort of Danish and Dutch men with severe hemophilia A (median follow-up, 3.6 years) compared outcomes of those who continued on prophylaxis with those of individuals who switched to episodic treatment. Although men who chose to switch to episodic treatment experienced more joint bleeding, the incidence (3.2 joint bleeds per year)10 was still considerably lower than the incidence of joint bleeds previously reported in other cohorts of adults with severe hemophilia who were managed with episodic treatment for their entire lives.

The multinational longitudinal Orthopaedic Outcome Study first supported initiation of prophylaxis in adults because of benefits to joints.11 Patients given full-time prophylactic therapy who were followed for 6 years had less progressive hemophilic arthropathy, as measured by physical and radiographic examinations.

Three recent studies in adults evaluated the efficacy of prophylaxis versus episodic treatment and compared the outcomes of both treatment regimens. Collins et al12 treated 20 adult patients (age, 30–45 years) sequentially with episodic treatment for 6 months and then crossed them over to 6 months of high-dose prophylaxis (20–40 IU/kg of rFVIII three times weekly). A comparison of the two interventions showed that patients with significant joint bleeding histories who were maintained on episodic treatment had no bleeding episodes once switched to prophylaxis. However, because this study was brief, it did not show improvement in joint function or QOL measures.

Valentino et al13 first treated 66 adult patients with 6 months of on-demand

### TABLE 2
Magnetic Resonance Imaging Findings in the Joint Outcome Study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Prophylactic therapy (n = 32)</th>
<th>Enhanced episodic treatment (n = 33)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients with primary outcome data</td>
<td>27</td>
<td>29</td>
<td>0.73</td>
</tr>
<tr>
<td>Number (%) with joint damage</td>
<td>2 (7)</td>
<td>13 (45)</td>
<td>0.002</td>
</tr>
<tr>
<td>Number (%) with no joint damage</td>
<td>25 (93)</td>
<td>16 (55)</td>
<td></td>
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</tbody>
</table>

Source: Manco-Johnson et al

### TABLE 3
ESPRIT Study Outcomes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Episodic therapy (n = 21)</th>
<th>Prophylactic therapy (n = 19)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Months in study, median (range)</td>
<td>81.9 (2–96)</td>
<td>84.4 (13–163)</td>
<td>NS</td>
</tr>
<tr>
<td>Age at study end, months, median (range)</td>
<td>148 (18–193)</td>
<td>154 (27–204)</td>
<td>NS</td>
</tr>
<tr>
<td>Hemarthroses/patient/year, median (range)</td>
<td>7 (0–68)</td>
<td>36 (0–117)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Number of patients with roentgenographic evidence of joint damage (%)</td>
<td>6 (29%)</td>
<td>14 (74%)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Number of factor VIII units infused</td>
<td>13,477,251</td>
<td>5,749,085</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Number of joint bleeding episodes</td>
<td>304,733</td>
<td>701,775</td>
<td>&lt; 0.01</td>
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</tbody>
</table>

NS = not significant

Source: Gringeri et al

### TABLE 4
Outcomes of Intermediate- vs High-Dose Prophylaxis for Patients with Severe Hemophilia

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<tbody>
<tr>
<td>Age at evaluation, years (range)</td>
<td>22.7 (20.4–25.3)</td>
<td>17.2 (15.2–20.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Joint bleeds/year (range)</td>
<td>2.5 (1–5.7)</td>
<td>0.5 (0.2–1.8)</td>
<td>0.050</td>
</tr>
<tr>
<td>Patients without joint bleeds</td>
<td>5%</td>
<td>25%</td>
<td>0.042</td>
</tr>
<tr>
<td>Clinical score (maximum = 90)</td>
<td>2 (0–5)</td>
<td>0 (0–4)</td>
<td>0.449</td>
</tr>
<tr>
<td>Pettersson score (maximum = 78)</td>
<td>10 (3.5–17.5)</td>
<td>4 (0–15)</td>
<td>0.750</td>
</tr>
<tr>
<td>Pettersson score = 0</td>
<td>11%</td>
<td>46%</td>
<td>0.560</td>
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</table>

Source: Fischer et al
treatment and then randomized these individuals to either 12 months of high-dose prophylaxis (20–40 IU/kg of rFVIII every other day) or a pharmacokinetically tailored arm in which patients were infused with 20–80 IU/kg of rFVIII every third day to maintain trough levels of > 1%. The annualized bleeding rates for both prophylactic groups were reduced by 99%, but there was no evidence of improvement in joint function. QOL measures for pain and physical function were improved at the end of the prophylactic treatment period. The third study, the multinational Trial to Evaluate the Effect of Secondary Prophylaxis with rFVIII Therapy in Severe Hemophilia A Adult and/or Adolescent Subjects Compared to That of Episodic Treatment (SPINART), was recently completed. In all, 84 patients were randomly assigned to treatment with a high-dose prophylactic regimen or episodic treatment. All patients underwent MRI examination of index joints on study entry and had yearly joint examinations and QOL assessments thereafter. At the end of the 3-year treatment period, the patients underwent another MRI examination. Patients in the prophylaxis arm were treated with 25 IU/kg of rFVIII three times per week (escalation by 5 IU/kg to a maximum of 30 or 35 IU/kg was allowed in patients with bleeding episodes after 1 and 2 years, respectively). For the episodic treatment arm, rFVIII replacement was left to the discretion of the treating physician.

An interim report (median follow-up, 1.4 years) showed a 93% decrease in bleeding rate among patients in the prophylaxis arm. There was also an implication that bleeding episodes in the prophylaxis arm were less severe. Data from target joint examinations and MRI and QOL assessments have not yet been reported.

Prophylaxis in adults decreases bleeding frequency by > 90% and the severity of bleeding episodes. There is early evidence that prophylaxis in adults improves arthropathy and QOL, but this still must be confirmed. Also, if evidence from the pediatric and pharmacokinetic studies is considered, many adults with hemophilia A probably can achieve bleeding control with less than the standard dose of 20–40 IU/kg of rFVIII three times per week, since the 48-hour trough levels with this dosing regimen have been 3–6 IU/dL in recent studies. Taking this approach also would help to decrease the discrepancy between the amounts of replacement factor used by the prophylaxis treatment groups and the episodic treatment groups. Given that each adult has a different bleeding history, hemophilic arthropathy, and factor activity level, dosing strategies should be individualized to optimize the costs of factor replacement and attain the best outcomes.

INHIBITORS AND CURRENT MANAGEMENT OPTIONS

Alloimmune inhibitory antibodies to FVIII (inhibitors) are serious complications of treatment with factor concentrates and develop in about 25%–30% of patients with severe hemophilia A. Inhibitors normally are detected fairly early in treatment after a median of 14–16 treatment days. The most significant risk factor that predisposes patients to developing inhibitors is the F8 gene mutation. Patients with large multixon gene deletions have a much higher rate of inhibitor formation than do those with missense mutations, and those with inversions have an intermediate risk of inhibitor formation. Race is another risk factor—black males have a higher rate of inhibitor development than do white males, although the etiology is unclear. Treatment-related factors also have been associated with inhibitor development. For example, early intensive factor exposure has been associated with increased inhibitor formation, and there may be some protection from inhibitor formation with the use of prophylaxis.

Immunogenicity of Therapeutic Products

Another potential risk factor involves plasma-derived products that are rich in von Willebrand factor (vWF) and less immunogenic than are recombinant factor products. Previous data were based on retrospective studies. The Research of Determinants of Inhibitor Development among Previously Untreated Patients with Haemophilia (RODIN) trial is the first study to prospectively observe whether some products are more immunogenic than others. Investigators collected prospective data on 574 previously untreated patients with hemophilia and followed them for 75 exposure days. There was no immunogenic difference between patients treated with plasma-derived products and those treated with recombinant factor. An analysis of individual factor products suggested that a second-generation, full-length recombinant factor might be related to a slightly higher relative risk for inhibitor formation, but the observational nature of this study cannot lead to definitive conclusions.

The ongoing Survey of Inhibitors in Plasma-Product Exposed Toddlers (SIPPET) is randomizing previously untreated patients to receive either rFVIII or plasma-derived FVIII product with vWF to determine whether some products are more immunogenic than others. Other risk factors for inhibitor formation reported in the literature include polymorphisms in immune regulatory genes and associations with specific human leukocyte antigen (HLA) class II alleles, but the evidence has been inconsistent, and any association with inhibitor formation remains unknown.

Immune Tolerance Induction (ITI)

Eradication of inhibitors uses ITI with frequent regular infusions of FVIII and works in about 60%–80% of cases (median time to tolerance, approximately 9–12 months). However, the optimal dose of
factor and optimal product (recombinant or plasma-derived) remain unknown.

The International Immune Tolerance Study looked at the issue of dose and randomized good-risk patients (defined as having a peak historical inhibitor titer of ≤ 200 BU/mL, starting titer level of ≤ 10 BU/mL before randomization, and age < 8 years at the time of randomization) to either a high-dose arm of 200 IU/kg daily of rFVIII or to a low-dose arm of 50 IU/kg three times a week. Inhibitor titers and half-life recovery studies were collected throughout the study. There was no difference in the time to tolerance induction and the rate of tolerance induction, although the time to negative inhibitor titer and time to normal recovery were slower in the low-dose arm.22 The trial was stopped early due to significantly more bleeds in the low-dose arm and to futility, since enrollment was not robust enough to determine equivalence between the arms.

Patients who cannot have their inhibitor eradicated and have a high inhibitor titer (> 5 BU/mL) are treated with bypassing agents, such as activated prothrombin complex concentrate (FEIBA), FVIII inhibitor bypass activity, or recombinant factor VIIa. Prophylactic treatment with either bypassing agent resulted in improved bleeding control when compared with episodic treatment.22,23

**POTENTIAL FUTURE TREATMENT OPTIONS**

New advances on the horizon include long-lasting factor concentrates soon to be available for both patients with hemophilia A and those with hemophilia B. Also, three active studies are examining gene therapy for patients with hemophilia B. These studies are employing adeno-associated virus (AAV) serotype 8 vector, which expresses FIX. Only the trial from the University College London/St Jude Children’s Research Hospital has reported data thus far, noting stable FIX levels of 1%–6% among 10 patients 8–40 months after treatment.24 In this study, higher doses of AAV-8 vector genomes yielded higher plasma FIX levels but were associated with abnormalities in liver function tests and immune responses to the AAV-8 capsids. These patients were managed with short courses of corticosteroids.

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Advances in Clotting Factors: From Bench to Bedside

Anthony Sung, MD
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Abstract  The history of clotting factors is inextricably tied to that of hemophilia. The development of recombinant factor VIII (rFVIII) and factor IX (rFIX) in the 1990s has resulted in hemophilia patients having a virtually normal lifespan and significantly fewer complications, such as joint and intracranial bleeding, following prophylactic infusions. However, these treatments are limited by the short-half lives of rFVIII and rFIX, meaning that patients must endure three- or four-times-weekly infusions. This report summarizes some of the exciting advances in the management of hemophilia that have been reported in recent years. They include the addition of polyethylene glycol (PEGylation) or fusion with another protein, such as immunoglobulin G or albumin, to decrease the frequency of infusions, provide prolonged protection from bleeding, limit the need for central venous access devices, and encourage patients to transition to a prophylaxis regimen. In addition, advances have been made in improving the safety and efficacy of long-lasting rFVIII proteins for hemophilia A and the development of alternative agents to treat hemophilia and anticoagulant-related bleeding (eg, warfarin reversal) and new oral anticoagulants (eg, direct thrombin or factor Xa inhibitors).

H emophilia is caused by functional deficiency of a single coagulation protein. Absence of such proteins—factor VIII (FVIII) in hemophilia A and factor IX (FIX) in hemophilia B—may lead to spontaneous internal bleeding with joint damage, intracranial hemorrhage, and death. Since the 1840s, transfusion of whole blood has been used to treat hemophilia-associated bleeding.1 It wasn’t until 1911 that FVIII was detected in plasma2 and 1937 when its role was described in hemostasis and the coagulation cascade3 (Figure 1). These advances led to the development of plasma transfusions in the 1940s, plasma concentrates in the 1950s, cryoprecipitate in the early 1960s, and freeze-dried FVIII products for storage and home use in the late 1960s.4

However, as use of blood products became more common, concerns began to arise about infectious contamination, highlighted by outbreaks of hepatitis C virus infection in the 1970s and human immunodeficiency virus (HIV) infection in the 1980s among hemophilia patients receiving pooled blood products. Concerns about infected blood products led to the development of recombinant FVIII (rFVIII; approved by the US Food and Drug Administration in 1992) and recombinant FIX (rFIX; approved in 1997).4

The use of these recombinant coagulation proteins has changed hemophilia care and increased the life-expectancy of patients with severe hemophilia from approximately 20 years in the 1970s to an essentially normal lifespan today. Long-term prophylactic factor replacement therapy also has reduced morbidity, decreasing the risk of joint damage and intracranial hemorrhage, and improved the quality of life for both children and adults with hemophilia.5-7 Recombinant factors are safe and provide independence from reliance on donor blood products.

However, use of recombinant factors is not without its challenges. These substances are expensive, costing more than $250,000 a year per adult in the United States.4 Because of their short half-life, they need to be administered every other day or several times a week, representing a significant time commitment and presenting problems with maintaining adherence. In addition, they require venous access, which often requires placement of a central venous access device (ie, port) and involves risks of sepsis and thrombosis. Finally, 25%-30% of hemophilia A and 3%-4% of hemophilia B patients develop alloantibodies to these recombinant factors, inhibiting their efficacy and resulting in renewed problems with bleeding.8 Whereas gene therapy is a potential solution,9 a cure is not yet available.

Thus, there is an urgent need for new, improved clotting factors to treat hemophilia patients and individuals with acquired bleeding problems due to administration of oral anticoagulants (eg, warfarin) who either have been given a supratherapeutic dose or have developed bleeding in conjunction with trauma and surgery. New oral anticoagulants, such as rivaroxaban and dabigatran, also present challenges to reversal and inhibition of bleeding events. Prothrombin complex concentrates (PCCs), fresh frozen plasma (FFP), anti-inhibitor coagulant complex

Dr. Sung is a Fellow in Hematology/Oncology at Duke University Medical Center, Durham, North Carolina.
Although the development of recombinant factors in the 1990s represented a tremendous advance in the treatment of hemophilia, efforts have been underway to circumvent problems associated with their short half-life and frequent dosing. Research teams have reported on the structure of FVIII and FIX and their mechanisms of action, allowing engineering of variants with improved half-lives. These methods include conjugation with polyethylene glycol (PEG) in a process known as PEGylation, use of PEGylated liposomes as a mechanism for sustained release, fusion with the crystallizable fragment (Fc) of the constant region of immunoglobulin (Ig) or albumin, and novel modifications to clotting factors. Although PEGylated liposomes have failed to demonstrate in vivo efficacy, other methods have shown considerable promise.

**MOLECULAR APPROACHES FOR IMPROVED CLOTTING FACTORS FOR HEMOPHILIA**

**Based on a presentation by Randal J. Kaufman, PhD, Professor, Del E. Webb Center for Neuroscience, Aging, and Stem Cell Research, and Director, Degenerative Disease Research Program, Sanford-Burnham Medical Research Institute, La Jolla, California.**

Although the development of recombinant factors in the 1990s represented a tremendous advance in the treatment of hemophilia, efforts have been underway to circumvent problems associated with their short half-life and frequent dosing. Research teams have reported on the structure of FVIII and FIX and their mechanisms of action, allowing engineering of variants with improved half-lives. These methods include conjugation with polyethylene glycol (PEG) in a process known as PEGylation, use of PEGylated liposomes as a mechanism for sustained release, fusion with the crystallizable fragment (Fc) of the constant region of immunoglobulin (Ig) or albumin, and novel modifications to clotting factors. Although PEGylated liposomes have failed to demonstrate in vivo efficacy, other methods have shown considerable promise.

**PEGylation**

PEGylation is achieved by covalently attaching PEG to residues on target proteins such as lysine or N-terminal amines. However, random PEGylation may reduce the activity of the protein, and product heterogeneity may result in inconsistent effectiveness.

More recently, site-directed PEGylation via attachment of PEG-maleimide to cysteine residues has improved results with many proteins, including tumor necrosis factor-alpha, monoclonal antibody Fab fragment, vascular endothelial growth factor-aptamer, epoetin beta, and interferon alfa. In this approach, missense mutations are introduced at surface residues of FVIII to incorporate cysteine residues for conjugation with PEG-maleimide. This allows selective modification at the desired location, so PEGylation does not interfere with protein function and ensures product homogeneity. To date, no long-term safety concerns have arisen with this method, including with PEGylated FVIII products developed by Bayer Healthcare (BAY 94-9027), Baxter (BAX 855), and Novo Nordisk (N8-GP).

Of note, PEGylated products may vary by site of PEGylation and type of PEG used. PEG molecules also may differ in size, with smaller molecules being more rapidly cleared than larger ones. In addition, tissue penetration may vary, with smaller molecules having greater permeability. With PEG molecules > 10 kDa, pinocytotic uptake into macrophages and Kupffer cells is increased; with PEG molecules > 30 kDa, kidney clearance is decreased; and with PEG molecules > 50 kDa, liver clearance is increased. Therefore, the pharmacokinetics and pharmodynamics—and safety and efficacy—of different PEG solutions vary.

In addition to improving half-life, PEGylation may reduce immunogenicity. PEGylation of L491C in the A2-domain of FVIII resulted in reduced inhibitory activity of a monoclonal antibody that reacts to this highly immunogenic region of FVIII. This finding has been supported by other studies with different PEGylated proteins. PEGylation also may limit the inhibitory effect of alloantibodies.

**Fusion Proteins**

By covalently fusing coagulation factors with proteins having a much longer
half-life, such as immunoglobulin G1 (IgG1) or albumin, the half-life of the resulting molecule may be extended. Furthermore, the Fc domain can improve the solubility and stability of the partner molecule, allowing easy and cost-effective purification by protein-A/G affinity chromatography. Support for this concept comes from the results of preclinical and phase I studies showing that fusion of the monomeric form of the IgG1 Fc to human FVIII, FIX, and factor VIIa (FVIIa) increases the half-life approximately 1.5- to 4-fold without impairing efficacy. No adverse events have been reported.20,21

Another fusion approach covalently attaches the clotting factor to albumin. Data from both preclinical and phase I studies have shown a 1.5- to 3-fold increase in plasma half-life and preserved efficacy with rFIX-albumin.22,23 This improvement was not found with recombinant albumin-bound FVIII, however, possibly because of interactions with von Willebrand factor (vWF).

Novel FVIII Molecules

An alternative strategy uses a recombinant, single-chain FVIII protein that prevents dissociation of the two chains of FVIII, resulting in higher affinity for vWF. Because vWF protects FVIII from proteolysis and clearance, this strategy improves the stability and half-life of FVIII, as supported by animal studies; this molecule now is being tested in human trials.24 Other strategies focus on reducing the immunogenicity of recombinant FVIII molecules by changing or substituting glycan and sulfyl groups.25

Novel Products in Preclinical Development

In addition to modifications of FVIII and FIX and administration of activated FVII (FVIIa), several additional novel approaches are in preclinical development, including factor Xase mimetics and inhibition of antithrombotic pathways.

FVIII enhances FIXa-mediated cleavage of FX; factor Xase mimetics accomplish this same function independent of FVIII. For example, a humanized bispecific monoclonal antibody to FIXa and FX showed both efficacy and a prolonged half-life (2 weeks) in a simian model of acquired hemophilia.26 The ultimate goal is to engineer such a product that combines efficacy, a prolonged half-life, and ease of administration via subcutaneous injection or oral delivery.

Whereas one approach of these new agents is to enhance clotting, the desired effect, hemostasis, also may be achieved by inhibiting antithrombotic pathways. For example, tissue factor pathway inhibitor (TFPI) inhibits FVIIa and FXa and helps to foster hemostasis. This concept was demonstrated by a monoclonal antibody to one of the Kunitz domains of TFPI in a rabbit model of hemophilia that resulted in decreased bleeding.27 Other TFPI inhibitors include nucleic acid aptamers,28 non-anticoagulant sulfated polysaccharide,29 and small antagonist peptides.30 Further studies will be needed to evaluate the relative merits of these approaches.

Antithrombin 3 (AT3) is another antithrombotic target. Alnylam Pharmaceuticals has developed a synthetic GalNAc-conjugated RNA interference (ALN-AT3) that suppresses liver production of AT3 mRNA. Pharmacologic studies have shown dose-dependent and reversible reduction of circulating AT3, which was associated with significantly increased thrombin generation and enhanced homeostasis in murine models of hemophilia.19 Use in wild-type animals induced thrombotic events and disseminated intravascular coagulation, whereas similar or even higher doses were well tolerated in mice with hemophilia A and B with no evidence of thrombosis.

Summary

Considerable advances in treating hemophilia and developing clotting factors have been reported over the past two decades. Current approaches focus on improving the safety, tolerability, and delivery of both existing recombinant factors and novel alternatives that target other aspects of the coagulation/antithrombotic pathways. These products are making their way from preclinical development in animal models to clinical trials in humans, offering the promise of even greater advances for patient care in the years to come.

LONG-LASTING RECOMBINANT FACTOR VIII PROTEINS FOR HEMOPHILIA A

Based on a presentation by Amy D. Shapiro, MD, Chief Executive Officer and Co-Medical Director, Indiana Hemophilia Treatment Center, Indianapolis, Indiana.

PEGylation and fusion of Ig or albumin to FVIII and FIX are exciting strategies for increasing the half-life of these coagulation factors that are now being tested in phase 1–3 clinical trials. This section will review these developments.

Clinical Studies of PEG-FVIII Conjugates

Three PEG-FVIII conjugates currently are being tested in clinical trials: B-domain deleted recombinant FVIII (PEG-BDD-rFVIII; BAY 94-9027; www.clinicaltrials.gov ID No. NCT01184820), PEGylated full-length rFVIII (BAX 855; www.clinicaltrials.gov ID No. NCT01736475), and glycol-PEGylated rFVIII (N8-GP; www.clinicaltrials.gov ID No. NCT01480180).

PEGylation can impair a protein’s activity if bound to the wrong site, so it is important that this link is produced in such a way that it maintains the protein’s original function. One method of ensuring site-specific PEGylation is site-specific mutagenesis, which introduces cysteine mutations on the surface of B-domain deleted FVIII. Bayer HealthCare used this method in designing BAY 94-9027: PEG was conjugated to surface-exposed cysteines of rFVIII to retain full in vitro activity and vWF binding.17

BAY 94-9027 was evaluated in a phase 1 study of 14 patients with severe hemophilia A.15 Seven patients were given 25 IU/kg twice weekly, and the other seven received 60 IU/kg once weekly. BAY 94-9027 was well tolerated and effective without causing serious adverse events or immunogenicity. The half-life was 19 hours, representing a 1.6-fold increase over the half-life of standard rFVIII (approximately 12 hours). Phase 2/3 studies are underway.

An alternative strategy was employed by Baxter scientists in the design of BAX
855, a 20-kDa PEGylated full-length rFVIII. This conjugation process resulted from the combination of an activated PEG reagent with accessible amino groups on FVIII; it was optimized to target and modify mainly the ε-amino groups of lysine residues. BAX 855 reportedly retains all physiologic properties of FVIII except binding to the low-density lipoprotein receptor-related protein clearance receptor; preclinical testing revealed normal activity and a prolonged half-life when compared with unmodified rFVIII.

N8-GP is a rFVIII with site-directed glycoPEGylation being developed by Novo Nordisk. It is synthesized in a Chinese hamster ovary cell line with a truncated B domain of 21 amino acids; the terminal sialic acid on an O-glycan structure in the truncated B-domain is replaced by a conjugated sialic acid containing a branched 40-kDa PEG, resulting in a protein with a single medium-weight PEG attached to the B-domain. When the coagulation system is activated, thrombin cleaves the B-domain with the attached PEG, resulting in activated FVIII.

N8-GP was evaluated in a dose-escalation study (25, 50, or 75 IU/kg/dose) in 26 previously treated patients with severe hemophilia A. It was well tolerated at all dose levels, and no patient developed an inhibitor or binding antibodies to FVIII or N8-GP. N8-GP exhibited a dose-linear pharmacokinetic profile with a mean half-life of 19 hours (range, 11.6–27.3 hours), representing a 1.6-fold increase in half-life over rFVIII in murine and canine models of hemophilia. Interestingly, the half-life of rFVIIIFc was comparable to that of rFVIII in neonatal Fc receptor (FcRn) knockout mice, supporting the role of the Fc fragment and interaction with FcRn in protecting the fusion protein from degradation.

A phase 1/2 study of rFVIIIFc in 16 patients with severe hemophilia who were given either 25 or 65 IU/kg rFVIII followed by an equal dose of rFVIIIFc showed a 1.5- to 1.7-fold increase in mean half-life (18.8 hours for both doses) for rFVIIIFc over that of rFVIII (12.2 hours for the lower dose and 11.0 hours for the higher dose). Both products had similar dose-dependent peak plasma concentrations. No drug-related adverse events, inhibitors, or severe bleeding was observed.

A phase 3, multicenter study of rFVIIIFc (A-LONG, www.ClinicalTrials.gov identifier NCT01458106) was completed recently. Treatment arms included individualized prophylaxis at 3- to 5-day intervals, weekly prophylaxis, and episodic (on-demand) treatment. Preliminary results from this study were presented by Mahlangu and coworkers at the 65th Annual Meeting of the National Hemophilia Foundation in October 2013 and are summarized by Dr. Holleh D. Husseinzadeh elsewhere in this edition of The Hemophilia Report. Once the data from this trial are fully compiled and analyzed, the study should provide valuable information on the safety and effectiveness of different strategies for prophylaxis of hemophilia A and on-demand treatment of bleeding episodes with rFVIIIFc. A similar study is ongoing in children (Kids A-LONG, www.ClinicalTrials.gov identifier NCT01458106).

**Summary**

PEGylation and Fc fusion are exciting strategies that may prolong the half-life of rFVIII; extend dosing intervals; and potentially improve compliance, access, and safety. Both PEGylated and Fc fusion products have a half-life of 18–19 hours, whereas the half-life of vWF also is 18 hours. Given that vWF is needed to stabilize and protect FVIII, it is possible that the half-life of vWF may represent a new limit to how far the half-life of VIII products may be extended. This remains a significant improvement over conventional products, and the final results of phase 3 studies such as A-LONG are eagerly anticipated.

**THE OLD AND THE NEW: PCCs, rFVIIa, AND LONG-LASTING COAGULATION PROTEINS**

Based on a presentation by Margaret V. Ragni, MD, MPH, Professor of Medicine, Division of Hematology/Oncology, University of Pittsburgh, and Director, Hemophilia Center of Western Pennsylvania, Pittsburgh, Pennsylvania.

PCCs contain combinations of clotting factors and proteins C and S. Four-factor PCCs (eg, Beriplex, Octaplex, Kcentra) contain factors II, VII, IX, and X, whereas three-factor PCCs (eg, Bebulin, Profilnine) contain factors II, IX, and X but little VII. These products initially were developed as bypassing agents to treat hemophilia patients with inhibitors to FVIII or FIX. These factors act downstream of FVIII and FIX (Figure 1), bypassing their activity. FEIBA is a formulation of PCCs that has activated clotting factors to enhance hemostasis. PCCs and FEIBA start working within minutes and carry a low risk of infectious transmission due to viral inactivation by filtration, nanofiltration, pasteurization, or solvent detergent treatment.

The substance known as rFVIIa also...
was developed as a bypassing agent to treat hemophilia-associated bleeding. It may produce a “thrombin burst” via activation of FIX, FX, and FII on the surface of activated platelets. Like PCCs and FEIBA, rFVIIa is expensive, and its use carries a significant risk of thrombosis.

To assess the efficacy of these hemostatic strategies, quantitative and qualitative laboratory measures of clot formation are needed. These analytic tools include the thrombin generation assay (TGA), thromboelastography (TEG), and rotational thromboelastometry (ROTEM), which provide a more comprehensive assessment of clot formation than do such standard assays as prothrombin time (PT) and activated partial thromboplastin time (aPTT).\(^\text{41}\) Important parameters of TGA include the lag time (the time to initiation of thrombin generation) and endogenous thrombin potential (area under the curve). Those of TEG include the rate of clot formation and strength and stability of the clot. For ROTEM, important parameters include the time to clot formation (clotting time), maximum clot firmness, and time to clot lysis.

**PCCs and rFVIIa in Surgery and Trauma (With or Without Warfarin)**

Preclinical studies in porcine liver laceration and spleen injury models provide in vitro and in vivo evidence that PCCs are more effective than rFVIIa in restoring thrombin generation and reducing blood loss.\(^\text{42,43}\) These results are supported by clinical studies of coagulopathic patients undergoing surgery with excessive bleeding requiring PCCs or rFVIIa.

In a study of patients undergoing cardiopulmonary bypass, three-factor PCCs (18.9–30.9 U/kg) reduced transfusion requirements to a greater degree than did rFVIIa (90–120 μg/kg).\(^\text{44}\) Another comparison study of three-factor PCCs (25 U/kg) with rFVIIa (90 μg/kg) in 85 traumatic brain injury patients revealed significantly greater reductions in red blood cell (RBC) and FFP requirements and a lower mortality among the PCC group.\(^\text{45}\) In a randomized trial of patients with acute major surgical hemorrhage who also received vitamin K, four-factor PCCs were superior to FFP; whereas other studies showed that the combination of FFP and PCCs may yield even better results.\(^\text{46,47}\)

At the same time, in coagulopathic trauma patients (half of whom were receiving warfarin), administration of three-factor PCCs (25 U/kg) rapidly corrected the international normalized ratio (INR) and reduced the RBC requirement; however, there was no survival benefit, as with most studies of rFVIIa in trauma.\(^\text{48}\) Controversy remains regarding rFVIIa use, given its high cost, lack of dosing guidelines, and thrombosis risk.

Another challenge of using PCCs and rFVIIa is determining the proper dose. Some studies have used algorithms that adjusted the dose based on INR (eg, three-factor PCC dosed at 25 U/kg for an INR of 2.0–3.9, 35 U/kg for an INR of 4.0–6.0, and 50 U/kg for an INR > 6.0), whereas others have used ROTEM testing to guide PCC therapy, and still others have used fixed doses.\(^\text{49,50}\) The optimal timing and frequency of administration also are unclear.

Of note, four-factor PCCs appear to be more effective than are three-factor PCCs.\(^\text{51}\) This may be due to consumption of FVII in cases of extensive surgery or trauma, bleeding, or warfarin use and the fact that three-factor PCCs are poor in FVII. When the INR > 6.0, three-factor PCCs may have little efficacy.\(^\text{50,51}\) Interestingly, in cases of warfarin reversal for acute bleeding, administration of 10–90 μg/kg of rFVIIa rapidly corrected the INR; unlike with four-factor PCCs, however, it seemed to do little to reduce bleeding.\(^\text{52}\) In parallel with these findings, ROTEM clot stability and clot lysis time in warfarin-treated patients appeared to improve more after treatment with PCCs than after use of rFVIIa.\(^\text{53}\)

**PCCs, rFVIIa, and the New Oral Anticoagulants**

New oral anticoagulants (thrombin and factor Xa inhibitors) have many advantages over warfarin, including no requirement for monitoring, few drug–drug interactions, and lower bleeding rates. However, reversal of these agents for life-threatening bleeding is complicated by the absence of an effective antidote. The half-life and duration of action of new oral anticoagulants are short. However, acute bleeding often occurs, and waiting for the drug to wear off is unacceptable.

Unfortunately, there is little evidence of the best approach to stop bleeding in patients on new oral anticoagulants. In preclinical studies, FEIBA, PCCs, and rFVIIa improved parameters such as bleeding time, PT, and aPTT, but there was poor correlation with the amount of blood loss.\(^\text{54,55}\) The best results appeared to follow the use of high-dose four-factor PCCs (eg, 50 U/kg) and FEIBA, with rFVIIa and FFP having little effect.\(^\text{56,57}\) Studies in healthy human volunteers appeared to support these findings, with reversal of abnormal TEG and ROTEM results seen with FEIBA (20–120 U/kg) and four-factor PCCs (50 U/kg); use of rFVIIa (20–120 μg/kg) was less effective.\(^\text{58}\) However, few data for these products exist in bleeding patients.

Novel antidotes to reverse new oral anticoagulants are being developed. They include an Xa congener that neutralizes Xa coagulation inhibitor function and a dabigatran-specific antidote (dAbi-Fab) that mimics thrombin structure (but not function) and binds to dabigatran with 350-fold greater avidity.\(^\text{60}\) These agents are still in the exploratory stage.

**Treatment Considerations**

To reverse warfarin-related bleeding or surgical and trauma-related bleeding, PCCs appear to be superior to rFVIIa and warfarin.\(^\text{61}\) The combination of PCC and FFP or PCC and rFVIIa may correct laboratory abnormalities such as INR even more rapidly,\(^\text{62}\) yet extreme caution must be exercised, given the significant thrombotic risk of each agent, and recommendations regarding combination therapy must await further studies. Furthermore, use of these agents should be avoided in patients with recent (< 3 months) thromboembolism.\(^\text{63}\) Even in the absence of thromboembolism, dosing should be judicious (eg, 25 U/kg of four-factor PCC for an INR of 2.0–3.9, 35 U/kg for an INR of 4.0–6.0, and 50 U/kg for an INR > 6.0).\(^\text{50,62}\) The lowest effective
dose of rFVIIa has not been established.

Regarding new oral anticoagulants, it is unclear whether PCCs or rFVIIa can reverse their effects. Caution should be exercised, since there apparently is little correlation between correction of laboratory abnormalities and reversal of bleeding, and dosing and monitoring are not established.8,63–65 New antidotes are under development but are experimental at this time. Transfusion support and surgical hemostasis should be provided, if indicated. Of note, dialysis with activated charcoal may be effective for dabigatran if initiated within 2–4 hours of ingestion; however, this may not be effective to reverse the effects of rivaroxaban, which is highly protein bound.65,66

**CONCLUSION**

Tremendous advances have been made in hemostasis over the past several years. Advances in clotting factors (eg, PEGylation, Ig or albumin fusion with FVIII or FIX) promise to improve hemophilia treatment by increasing factor half-life, decreasing the frequency of infusions, and potentially improving compliance and access while decreasing the risk of bleeding complications. Other novel agents (eg, TFPI, AT3 inhibitors) are also very exciting. Meanwhile, FEIBA, PCCs, and rFVIIa may help decrease life-threatening bleeding for hemophilia patients and those on warfarin or new oral anticoagulants who experience surgical or traumatic bleeding. As with all these agents, caution must be exercised, given the risk of upsetting the balance between hemostasis and thrombosis. Nonetheless, the coming years promise major advances in clotting factor treatments.

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Hemophilia A and B: Disease Differences and the Use of Prophylactic Therapy

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**Abstract** The treatment algorithm for hemophilia has evolved substantially since the advent of factor replacement. Prophylaxis is now the standard of care for children with hemophilia A and B. However, unresolved treatment decisions, including the age to start treatment, optimal dosing, and continuation of prophylaxis into adulthood, remain. Additionally, exciting new areas of managing hemophilia are on the horizon, including treatment with new recombinant coagulation factors that have prolonged half-lives and potentially curative treatments.

Hemophilia A and B are X-linked, recessive disorders caused by deficiency or absence of coagulation factors VIII (FVIII) and factor IX (FIX), respectively. They are classified into severe (<1%), moderate (1%–5%), or mild (>5% to <40%) categories according to coagulation factor activity.

Hemophilia A is four times more common than is hemophilia B. Because they are clinically indistinguishable, they historically were believed to represent the same disease. It was not until 1952 that hemophilia B was considered to be a separate entity. The two types share many similar components (eg, prolongation of activated partial thromboplastin time, recurrent hemarthroses), but they have some distinct differences (eg, frequency of inhibitor development, type of genetic mutations). For example, hemophilia B is caused by less severe gene mutations than hemophilia A and is characterized by more missense mutations than null mutations. As a result of these differences, only 35% of patients with hemophilia B have severe disease, compared with 45% of those with hemophilia A.

The subtypes also differ in coagulation factor pharmacokinetics—the half-life of FIX is 18 hours and of FVIII is 11 hours. As a result, post-infusion levels after FIX administration are sustained for longer periods, thereby reducing the risk of recurrent bleeding. The reduced incidence of recurrent hemarthrosis may contribute to a threefold difference in the need for total joint arthroplasty in people with hemophilia B as compared with those diagnosed with hemophilia A. Nonetheless, the treatment approaches are similar—and most clinical research for treatment bundles the two diseases.

During a Baxter Healthcare–sponsored symposium offered at the 65th Annual Meeting of the National Hemophilia Foundation, experts described the history of hemophilia B therapy and looked toward its future. The panelists included Amy Shapiro, MD, and Natalie Duncan, MPH, of the Indiana Hemophilia and Thrombosis Center in Indianapolis; Erik Berntorp, MD, PhD, of the Malmö Centre for Thrombosis and Haemostasis at Lund University in Lund, Sweden; and Marion Koerper, MD, of the University of California, San Francisco School of Medicine in San Francisco, California.

**E Volution of Current Treatment Approaches**

Multiple factor replacements are available in the United States to treat hemophilia B (Table 1). At one time, the initial management strategy for hemophilia patients was on-demand treatment to provide factor in response to an acute episode of bleeding or trauma. On-demand factor replacement remains the treatment of choice for all patients with mild disease and most of those with moderate disease. In the 1960s, however, clinicians first thought of “converting” individuals with severe disease to moderate manifestations using scheduled prophylactic treatment.

**Preventative Measures**

Prophylaxis aims to prevent bleeding episodes before they result in arthropathy and life-threatening hemorrhage. Prophylaxis, however, is expensive and inconvenient, because it requires frequent dosing. Therefore, decades of research were necessary before prophylaxis became the standard of care for children with severe hemophilia.

Some initial evidence to support the prophylactic approach arose from large observational studies. One large study of 156 patients with hemophilia compared Swedish and Norwegian individuals di-
agnosed with the disease. Clinicians in Sweden have long used prophylaxis as the standard of care, whereas those in Norway had embraced an on-demand treatment approach. Patients given on-demand treatment had five times more episodes of bleeding and used more resources outside of the healthcare setting, but the prophylaxis group had significantly higher annual factor-concentrate consumption.

A similar study compared hemophilic patients in the Netherlands, who were given intermediate-dose prophylaxis, with patients in Sweden, who were given high-dose prophylaxis, and France, who were given on-demand treatment. Higher rates of bleeding and arthropathy were noted among the on-demand treatment group; however, there was minimal reported clinical improvement between intermediate-dose and high-dose prophylaxis. Costs for on-demand and intermediate-dose prophylaxis were similar, whereas high-dose prophylaxis was significantly more expensive.

Despite these and other observational data, authors of a 2006 Cochrane review found insufficient evidence to support prophylaxis and called for randomized, controlled trials. Shortly thereafter, a randomized, controlled trial showed that 25 IU/kg of prophylactic factor replacement given every other day decreased bleeding episodes and hemarthroses in patients with hemophilia A. The prophylaxis group used twice as much factor annually at a higher financial cost.

Subsequent studies have upheld the positive clinical impact of prophylaxis. The latest Cochrane review has recommended prophylaxis to preserve joint function. According to this review, evidence of benefit from prophylaxis in patients with preexisting joint damage is insufficient. Thus, early initiation of factor replacement is needed for children, although the exact age for treatment initiation is uncertain.

### Timing and Dosing Are Everything

Multiple methods of prophylaxis have been defined (Table 2), and the best strategy to follow has been hotly debated. An early start is important, since it can lessen future degenerative joint disease. However, starting treatment in toddlers aged 1–2 years is not without its challenges. For example, complication rates associated with central catheter placement brings venous access into question. The model used at the Malmö Hemophilia Center in Sweden (Figure 1) details one approach for primary prophylaxis of hemophilia B.

Optimal dosing is another unresolved component of prophylaxis. Internationally, different treatment approaches have been proposed (Table 3). The most expensive scheme, the high-dose Swedish prophylactic regimen, previously was considered the best to prevent joint disease and bleeding. More recently, a retrospective analysis of the Dutch intermediate-dose program compared with the much more costly Swedish high-dose regimen concluded that the latter offered minimal added benefit. The alternative pharmacokinetic approach remains promising, but there is insufficient evidence that factor level peaks and troughs predict bleeding risk. In clinical practice, prophylactic regimens and dosing usually are tailored to patients’ individual needs.

Lower costs have sparked interest in low-dose regimens, especially when they are used in resource-poor settings. Wu et al. demonstrated that low-dose prophylaxis used in China reduced the number of bleeding episodes and improved joint function over 12 weeks. Patients with hemophilia A were given 10 IU/kg of FVIII twice weekly, and those with hemophilia B were given 20 IU/kg of FIX once weekly. In the Swedish study, however, the average prophylactic dose was 25–40 IU/kg of FVIII given three times a week for patients

### TABLE 1

<table>
<thead>
<tr>
<th>CURRENT FACTOR IX REPLACEMENT PRODUCTS AVAILABLE IN THE UNITED STATES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product</strong></td>
</tr>
<tr>
<td>AlphaNine SD</td>
</tr>
<tr>
<td>BeneFIX</td>
</tr>
<tr>
<td>Mononine</td>
</tr>
<tr>
<td>Rixubis</td>
</tr>
</tbody>
</table>

**Source:** Shapiro

### TABLE 2

**Definitions of Prophylaxis Used by the European Pediatric Network for Hemophilia Management**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary prophylaxis A</td>
<td>Regular continuous treatment started after the first joint bleed and before the age of 2 years</td>
</tr>
<tr>
<td>Primary prophylaxis B</td>
<td>Regular continuous treatment started before the age of 2 years without previous joint bleed</td>
</tr>
<tr>
<td>Secondary prophylaxis A</td>
<td>Regular continuous (long-term) treatment started after two or more joint bleeds or at an age &gt; 2 years</td>
</tr>
<tr>
<td>Secondary prophylaxis B</td>
<td>Intermittent regular (short-term) treatment, because of frequent bleeds</td>
</tr>
</tbody>
</table>

**Source:** Donadel-Claeyssens

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**FIGURE 1** Approach to primary prophylaxis of hemophilia B, as practiced at Malmö Hemophilia Center in Sweden. Adapted, with permission, from Berntorp.
with hemophilia A and 25–40 IU/kg of FIX twice a week for those with hemophilia B. The Chinese low-dose prophylactic approach reduced costs, but whether it prevented long-term arthropathy-related disability remains unclear, since a moderate number of joint bleeds remained.

**How Much Is Too Much?**

Prophylaxis has dramatically improved quality of life and reduced arthropathy in children with hemophilia. Whether and when to stop prophylaxis remain controversial. Presenting data from the HUGS (Hemophilia Utilization Group Study) VB trial, Koerper revealed that two thirds of children and one half of adults with hemophilia B at one US hemophilia treatment center were on prophylaxis. Early retrospective data did not show a significant difference in arthropathy scores when on-demand therapy and prophylactic treatment were compared in adults. Definitive recommendations for prophylaxis in adults will require long-term, prospective, randomized trials to adequately assess outcomes.

### CHALLENGES IN USING PROPHYLACTIC REGIMENS

One barrier to standard use of the prophylactic regimen is the cost. Prophylaxis has become less expensive, as it has become the standard of care for children with hemophilia in the United States; however, patient adherence to the regimen remains challenging.

With currently available formulations, patients with hemophilia B require prophylaxis two to three times a week. Only 60% of hemophilia patients report infusing at least three fourths of the recommended factor, most commonly missing doses because of their complexity and the time commitment. Paradoxically, the ability of prophylactic factor replacement to completely eliminate symptoms decreases adherence.

This problem, however, is not limited to patients in the United States. A Chinese study examining prophylaxis versus on-demand treatment found that although factor was provided at no cost, adherence rates lagged. The primary reason for this phenomenon was that patients and their families did not fully understand the importance of therapy. Recent studies by the same group showed significant barriers to prophylaxis in areas without dedicated hemophilia treatment centers.

### IMPROVING ADHERENCE

Several methods have been developed to improve adherence rates. Two modifiable factors that influence adherence are maintenance of a good relationship between the patient and the healthcare provider and the patient’s positive belief in the necessity of treatment. With regard to the latter, families reported that expanded health education best facilitated adherence. Novel treatment approaches that may help improve adherence rates are being developed.

### NOVEL APPROACHES TO HEMOPHILIA TREATMENT

Current hemophilia treatments have improved the quality of life and longevity of patients with hemophilia dramatically, yet limitations such as the inconvenience of frequent dosing and its substantial cost remain. Prolonging the half-life of factor replacement may lower costs and decrease complications and inconvenience related to frequent venous access.

The half-life of therapeutics can be extended by using PEGylation, which involves covalent attachment of polyethylene glycol (PEG) polymer chains to liposomes. Another technique involves recombinant fusion proteins, in which FIX is attached to the crystallized fragment region of immunoglobulin G. In these trials, bleeding-free intervals or blood clotting times have been prolonged significantly. Currently, at least three phase 3 clinical trials of long-acting FIX, and many more trials in phase 1 or phase 2, are ongoing.

An alternative approach is gene therapy. Because a small increase in factor level can significantly improve bleeding rates, this method offers a potential cure for hemophilia B. treated six patients with severe hemophilia using a viral vector that expressed FIX. All patients had less need for factor replacement, and four patients stopped using prophylaxis completely. Additional trials of this extremely promising area of research are ongoing.

### REFERENCES

5. Shapiro A. Treatment update on hemophilia B. Presented at the 65th Annual Meeting of the National Hemophilia Foundation; October 3–5, 2013; Anaheim, California.

### TABLE 3

Dosing Strategies for Long-Term Prophylaxis

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Principle</th>
<th>Relative cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dutch regimen (intermediate-dose)</td>
<td>15–25 IU/kg 2–3 times/wk, starting early after the occurrence of joint bleeds</td>
<td>Moderate</td>
</tr>
<tr>
<td>Traditional Swedish dosing regimen (high-dose)</td>
<td>25–40 IU/kg 2–3 times/wk, starting before the occurrence of joint bleeds</td>
<td>Most expensive</td>
</tr>
<tr>
<td>Alternative Swedish dosing regimen (pharmacokinetic dosing)</td>
<td>Individualized to patient needs, starting with a high dose and followed by a reduction in dose and lengthening of the interval between doses</td>
<td>Least expensive</td>
</tr>
<tr>
<td>Canadian regimen (dose escalation)</td>
<td>50 IU/kg weekly, starting early after the occurrence of joint bleeds and intensified stepwise depending upon bleeding frequency</td>
<td>Less expensive</td>
</tr>
</tbody>
</table>

Source: Berntorp and Shapiro15

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Anna Chalmers, MD  
**Hemophilia A and B: Disease Differences and the Use of Prophylactic Therapy**


Genetics of Hemophilia: The Role of Genetic Testing and the Use of Genetics to Guide Treatment

Anna Chalmers, MD
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Abstract  Advances in genetics have led to the isolation and characterization of the genes responsible for hemophilia. The role of hemophilia treatment centers now commonly includes genetic counseling and testing. The ability to individually specify genotype in affected patients may lead to more effective individualized treatment plans.

Hemophilia A (classic hemophilia) and B (Christmas disease) are the two main types of inherited bleeding disorders that hamper the blood-clotting process. 1,2 Although they result from mutations in different genes, they have similar clinical courses. Hemophilia A occurs in up to 1:5,000 males around the world, whereas hemophilia B occurs in about 1:20,000 newborn males globally.3

During a Biogen Idec–sponsored symposium offered at the 65th Annual Meeting of the National Hemophilia Foundation, experts discussed the genetics of hemophilia A and B and presented talking points that they often share with their patients, including those addressing family planning and the care of pregnant patients with hemophilia. The panel of speakers included Barbara Konkle, MD, Professor of Medicine at the University of Washington School of Medicine, Director of Translational Research, and Medical Director of the Hemostasis Reference Laboratory at the Puget Sound Blood Center in Seattle, Washington; Michelle Alabek, MS, CGC, of Norton Cancer Institute Genetic Counseling Services at Norton Suburban Hospital in Louisville, Kentucky; and Jennifer Maahs, PNP, MSN, of the Indiana Hemophilia and Thrombosis Center (IHTC) in Indianapolis, Indiana.

The panelists also discussed the genetics of hemophilia A and B. Congenital deficiencies in factors VIII (FVIII; hemophilia A) and IX (FIX; hemophilia B) result from mutations of the F8 and F9 genes on the X chromosome, respectively. Males have only one X chromosome, so a single altered copy of one of these genes in each cell is enough to cause hemophilia. Females, on the other hand, carry two X chromosomes, so women need a mutation in both copies of the gene—one inherited from their mother and the other from their father—to develop the disease. Consequently, hemophilia is exceedingly rare in females.

Many types of mutations of F8 and F9 can lead to hemophilia. Further, the type of mutation can predict disease severity. Deletion and insertion mutations and mutations that cause premature termination of synthesis usually cause severe disease. Certain missense mutations also can lead to severe hemophilia. More commonly, missense mutations cause mild or moderate disease; in fact, 90% of patients who have mild-to-moderate hemophilia A have missense mutations. The majority of patients with hemophilia B also have missense mutations.

In hemophilia A, the most common genetic mutation is the intron 22 inversion mutation, which accounts for approximately 45% of all cases.4 This mutation arises from a folding over of the tip of the X chromosome, which leads to a homologous recombination. When the tip unfolds, some exons (nucleotide sequences) are oriented in the opposite direction, or inverted.4 Interestingly, most inversions originate during male meiosis (ie, in the maternal grandfather).5 As a result, almost all mothers of patients with inversion mutations are carriers of hemophilia.

Who Should Be Tested and How?

Understanding the most frequently encountered genetic mutations in patients with hemophilia can help to guide diagnostic testing. Patients with a severe FVIII deficiency are first screened for the intron 22 inversion mutation (Figure 1).4 If they test negative for this mutation, they undergo gene analysis. Patients with mild disease and hemophilia B undergo gene analysis. Currently, the Medical and Scientific Advisory Committee (MASAC) for the National Hemophilia Foundation recommends that all patients...
with hemophilia have genotype testing. An ongoing initiative is creating a comprehensive de-identified database of these mutations.

Appropriate family members of hemophilia patients also can undergo carrier testing. Analysis of data from patients undergoing pretesting counseling found that approximately 30% of cases of hemophilia are sporadic. Despite this finding, most carriers are unaware of their status and require supportive counseling.

Most carriers are asymptomatic. In unusual circumstances, such as cases of Turner syndrome, some carriers may have low enough levels of a blood factor to cause clinical bleeding. For most patients, though, carrier status only affects preconception counseling.

Testing a Carrier

To test a possible carrier, it is best to target a known mutation. If the mutation is unknown, then the approach is similar to that used for testing patients with hemophilia.

Patients are first screened for intron 22 inversion mutations, the most common overall cause of unspecified hemophilia. If the result is negative, then the whole gene is screened. Once carrier status is confirmed, patients can be counseled regarding family planning options, including preimplantation genetic testing. Patients who are carriers and pregnant with male fetuses need to be followed by a multidisciplinary team of specialists throughout pregnancy to address any possible perinatal testing and ensure a safe delivery.

USE OF GENETICS TO GUIDE TREATMENT

Traditionally, analysis of factor levels and clinical presentation have guided the treatment course for hemophilia. However, a small percentage of patients with severe disease, as defined by coagulation factor level, have a milder clinical presentation. Therefore, there has been some interest in using genetic information to help guide management of the disease. For example, based on the results of genetic testing, a healthcare provider may consider inserting a central line earlier in some patients or delaying primary prophylaxis in others.

To take a closer look at the value of genetic testing, Carcao and colleagues examined 621 previously untreated patients and classified them according to whether they carried null or non-null mutations. Patients with null mutations experienced their first hemorrhage at a younger age, even though the difference was only 2 months. In the future, genetic characterization may allow individualized treatment.

Characteristics of Hemophilia A (Classic Hemophilia)

- Caused by a mutation in the gene encoding coagulation factor VIII (F8) on chromosome Xq28
- Clinically heterogeneous, with severity dependent upon plasma levels of factor VIII (FVIII)
  - Mild: FVIII levels 6%–30% of normal; excessive bleeding only after trauma or surgery; experienced by 40% of patients
  - Moderate: FVIII levels 1%–5% of normal; experienced by 10% of patients
  - Severe: FVIII levels < 1% of normal; an average of 20–30 episodes/year of spontaneous and/or excessive bleeding, particularly into the muscles and joints, may occur after minor trauma; experienced by 50% of patients
- Joint involvement causes swelling, pain, decreased function, and degenerative arthritis.
- Muscle involvement causes necrosis, contractures, and neuropathy by entrapment.
- Hematuria (occasional) is usually painless.
- Intracranial hemorrhage is uncommon but can occur after mild head trauma and lead to severe complications.
- Persistent bleeding from tongue or lip lacerations may occur.

Source: McKusick and Kniffin

Characteristics of Hemophilia B (Christmas Disease)

- Caused by a mutation in the gene encoding coagulation factor IX (F9) on chromosome Xq27.1
- Phenotypically indistinguishable from hemophilia A; on blood testing, however, hemophilia B is associated with a prolonged activated partial thromboplastin time and a normal prothrombin time.
- A distinction has been made between cross-reactive material (CRM)-negative and CRM-positive hemophilia B mutants based on the detection of F9 antigen in plasma even in the presence of decreased F9 activity. About 90% of hemophilia B patients are CRM-negative.
- Treatment for factor IX deficiency involves replacement of the missing factor by transfusion of plasma from a healthy person or infusion of a recombinant factor IX product.
- A subset of patients develops immunoglobulin G antibodies against normal factor IX, which complicates therapy.

Source: McKusick and Harnosh
Development of Inhibitors

Most patients with the inhibitor phenotype have large alterations of the F8 or F9 gene (e.g., large deletions, nonsense mutations, frameshift mutations). A small percentage of patients with the inhibitor phenotype can develop anaphylaxis to replacement factor, a life-threatening, difficult-to-manage clinical situation.

Thorland et al.10 genotyped eight patients with hemophilia B who had experienced anaphylaxis when exposed to FIX therapy and compared them with patients with severe disease. Those who had complete gene deletions were at the highest risk of developing anaphylaxis.

SUMMARY

Recognition that patients with hemophilia are at high risk may impact healthcare decisions. For example, patients at high risk of anaphylaxis may need to be infused in the supervised clinic setting instead of receiving treatment at home. Genetic testing remains expensive, but used judiciously, it has the potential to improve the management of patients with hemophilia.

REFERENCES


Expanding Therapeutic Options for Hemophilia A and B: Results of Recent Trials and Research

Holleh D. Husseinzadeh, MD
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Abstract  An overview of current research on bleeding disorders illustrates the vast spectrum of investigative interest. During the 2013 Annual Meeting of the National Hemophilia Foundation, researchers presented the results of a broad variety of studies related to a basic understanding of these diseases in specific patient populations, preclinical experimentation, and the clinical use of current and promising future prophylactic and therapeutic modalities.

During the 2013 Annual Meeting of the National Hemophilia Foundation, researchers presented the results of their studies on development of novel therapeutics, gene-transfer therapies, and new technologies for lengthening factor half-lives and battling inhibitors against factor replacement concentrates. In addition, experts in hemophilia and its treatment discussed findings on improving outcomes following total knee and hip replacement in affected patients and the influence of obesity on compliance with prophylactic infusion regimens.

PRECLINICAL RESEARCH
Phospholipid-Binding Affinity of Factor IX (FIX)

Deficiency of FIX causes hemophilia B. Replacement of FIX with plasma-derived or recombinant factor concentrates is the key step in preventing bleeding episodes but is hampered by the short half-life (approximately 24 hours) of currently available FIX products, requiring frequent infusion. Consequently, researchers are investigating ways to extend their half-life without jeopardizing their safety or efficacy.

FIX is a member of a family of vitamin K–dependent proteins that contain multiple gamma-carboxyglutamic acid (Gla) residues in homologous amino-terminal Gla domains. Gla allows these proteins to bind to calcium and phospholipids. Of the vitamin K–dependent coagulation factors, FIX has one of the lowest phospholipid-binding affinities, which is thought to shorten its half-life.

Harvey and coworkers1 purposefully mutated three specific amino acids (Y1A, G4Y, and K5L) in the amino-terminal of the FIX protein that were expressed in a human embryonic kidney cell line (K293). Effects of these changes on phospholipid-binding affinity were examined individually and in combination.

The “triple mutant” resulted in a 27-fold increase in phospholipid-binding affinity. The mutation Y1A showed no benefit over wild-type protein, whereas the mutation G4Y resulted in a 2.5-fold increase in binding affinity alone and a 4.3-fold increase when combined with the mutation Y1A. Thus, synergy of the different mutations, G4Y and Y1A, was suggested. The mutation K5L led to an improvement in phospholipid-binding affinity that was not affected by mutations at the other two sites.

The ability to modify the binding affinity of FIX proteins to phospholipid membranes has attractive implications for the development of novel replacement coagulation factors with a significantly longer half-life, resulting in less-frequent dosing.

Binding of Factor VIII (FVIII) to von Willebrand Factor

Binding of FVIII to von Willebrand factor plays an important role in the biologic activity and clearance of FVIII and influences its presentation to the immune system.

Clara et al2 compared the affinity of a novel, recombinant single-chain factor VIII (rFVIII-SingleChain; CSL627) with that of recombinant full-length FVIII (rFVIII) to purified, plasma-derived von Willebrand factor (pdVWF) in vitro. The affinity of CSL627 for pdVWF was significantly higher than that of commercially available, full-length rFVIII proteins. Dissociation-rate constants of the two molecules were comparable, which suggested similar bioavailability. Other in vitro characteristics of the novel agent (FVIII enzymatic activity, thrombin generation, and ability to bind to phospholipids) were comparable to those of full-length rFVIII.

Systemic availability and mean residence time were higher in hemophilia A mice treated with single doses of CSL627 compared with full-length rFVIII. Addi-
tionally, a decrease in clearance rate and an increase in terminal plasma half-life were observed in mice that had received CSL627 versus full-length rFVIII. The volume of distribution and in vivo recovery of the two molecules were similar.3

This novel recombinant molecule is a promising new agent being developed to treat hemophilia A. Its higher infinity to pdVWF may delay its elimination from plasma and positively affect its systemic availability.

## CLINICAL TRIALS

BAX 855 is a PEGylated recombinant FVIII product designed to prolong the half-life of commercially available octocog alfa via covalent binding of polyethylene glycol (PEG) moieties. Covalent attachment of PEG moieties decreases the systemic clearance of rFVIII, theoretically without affecting its efficacy and safety.

Bevan and others3 assessed the efficacy and safety of BAX 855 in previously treated patients with severe hemophilia A and compared the pharmacokinetics of this novel compound with those of octocog alfa. In all, 24 patients with hemophilia A who had been exposed to rFVIII for at least 150 days and who had no history of inhibitors were enrolled into this multinational, open-label, phase 1 study. A total of 19 participants completed the study. Patients first received a single dose of octocog alfa and then underwent pharmacokinetic evaluation. After a washout period, the patients received either 30 IU/kg (cohort 1) or 60 IU/kg (cohort 2) of BAX 855, followed by 7 days of pharmacokinetic monitoring. Investigators followed the patients for 4 weeks after they received BAX 855 for safety assessments (adverse events, changes in vital signs, and laboratory test results) and immunogenicity (development of FVIII inhibitors; binding antibodies to FVIII, BAX 855, and PEG).

The mean half-life of rFVIII was 1.4- to 1.5-fold higher for BAX 855 than for octocog alfa in cohorts 1 and 2, respectively. Measurement of thrombin generation showed that mean peak thrombin concentration was increased above baseline for more than 120 hours after infusion of BAX 855 at 60 IU/kg (cohort 2). Other pharmacokinetic parameters were similar to, or better than, those of octocog alfa. No serious or treatment-related adverse events were recorded in either cohort. In addition, none of the patients developed FVIII inhibitors or experienced thrombotic or allergic events or significant changes in their vital signs or laboratory measurements.

These results suggested that BAX 855 has a longer half-life than that of octocog alfa, potentially allowing less-frequent dosing while offering similar efficacy and safety. Investigators affiliated with the PROLONG-ATE study currently are enrolling patients for phase 2/3 studies of BAX 855.

The exciting results of this trial represented the first successful gene-transfer therapy in hemophilia resulting in long-term expression of a deficient F9 gene. The trial is reopening and is actively recruiting adults (age > 18 years) with severe hemophilia B.

### Extending Factor Half-Life with Fusion Protein Technology

Currently available formulations of rFVIII given to treat hemophilia A have short half-lives (approximately 12 hours). Thus, to prevent and treat bleeding episodes, frequent injections of these agents are needed. Combined use of crystallizable fragment (Fc) fusion protein technology with rFVIII results in an rFVIIIFc fusion protein with an extended half-life (mean, 19 hours). Mahlangu et al5 discussed the A-LONG study, which assessed the efficacy, safety, and pharmacokinetics of rFVIIIFc when given for the prophylaxis and treatment of hemophilia A.

Study participants included 165 previously treated males ≥ 12 years of age who had severe (≤ 1 IU/dL) hemophilia A. Patients in arm 1 received individualized prophylaxis with pharmacokinetic-driven adjustment of dose and interval, those in arm 2 received weekly prophylaxis at a constant dose, and those in arm 3 received episodic treatment as needed for bleeding
events. Dosing intervals achieved in arm 1 were ≥ 3.5 days, with 30% of patients achieving a dosing interval of 5 days during the last months of the study. The average number of bleeding episodes was 1.6 in arm 1, 3.6 in arm 2, and 33.6 in arm 3. Administration of just one injection resolved 87.3% of bleeding episodes. None of the participants developed inhibitors to rFVIIIFc. Excellent or good control of bleeding was seen in patients who received rFVIIIFc and underwent major surgery during the study. Adverse events were similar to those expected among the general hemophilia A population; no patient developed inhibitors to rFVIIIFc. This was the first clinical study of rFVIIIFc fusion protein in patients with hemophilia A. Some prolongation of the dosing interval and other preliminary results suggested that rFVIIIFc offers a safety profile similar to that of existing recombinant factor agents.

**Examining Recombinant Fc Fusion Protein Technology in Hemophilia B**

Available FIX replacement products for patients with hemophilia B have relatively short half-lives. As a result, they require frequent self-injection, which is often disruptive for patients and may lead to decreased compliance and hemophilia-related complications. A novel recombinant FIX Fc fusion protein (albumin; rFIXFc) with an extended half-life has been developed to help reduce the burden of frequent prophylactic injections.

In the B-LONG study, one of the largest clinical studies conducted in patients with hemophilia B, investigators assessed the safety, efficacy, and pharmacokinetics of rFIXFc given to prevent and treat bleeding episodes. The study involved 123 previously treated males ≥ 12 years of age who were diagnosed with severe hemophilia B (< 2 IU/dL). Patients received weekly prophylaxis with pharmacokinetics-driven rFIXFc dose adjustment (arm 1), individualized interval prophylaxis with pharmacokinetics-driven interval adjustment (arm 2), on-demand administration for acute bleeding episodes (arm 3), or factor management for anticipated surgery (arm 4).

The mean half-life for rFIXFc was 82.1 hours. In arm 2, the dosing interval in all patients was ≥ 7 days, but over one half of these patients were able to be maintained on a dosing interval ≥ 14 days. The median number of bleeding episodes per year was 3.0 in arm 1 and 1.4 in arm 2; these rates were much lower than the median 17.7 episodes per year observed in arm 4. A single dose of rFIXFc was sufficient to control 90.4% of bleeding episodes in arm 3. In arm 4, bleeding control was rated as good or excellent with all major surgeries in study participants. rFIXFc was well tolerated and caused no major side effects or development of inhibitors in study participants.

Thus, the preliminary results from this first clinical study of an rFIXFc fusion protein in hemophilia B patients showed this agent to be well tolerated. Its use led to a markedly increased dosing interval, which may lead to greater compliance, decreased hemophilia-related complications, and improved quality of life.

**Prophylaxis in Hemophilia with Inhibitors**

Factor Eight Inhibitor Bypass Activity Nanofiltered (FEIBA NF; anti-inhibitor coagulant complex) is an activated prothrombin complex concentrate used to control spontaneous bleeding episodes or to cover surgical interventions in patients with hemophilia A or B who have developed inhibitors to factor replacement concentrates. Antunes and others investigated whether prophylactic use of FEIBA NF could be as beneficial and safe as on-demand use.

In this prospective, randomized study, 36 patients with hemophilia A or B who had developed inhibitors against replacement factors were randomized to receive either prophylaxis or on-demand regimens of FEIBA NF for 12 months. In all, 17 patients were randomized to receive a prophylactic regimen of 85 ± 15 U/kg of FEIBA NF every other day. The remaining 19 patients were given on-demand FEIBA NF as directed by their treating physicians for acute bleeding events.

The results are summarized in Table 1. Patients receiving FEIBA NF prophylactically experienced 196 bleeding episodes, whereas those receiving it on demand had 629 bleeding episodes. The median annualized bleeding rates in the prophylactic FEIBA NF arm were significantly lower than those in the on-demand arm (7.9 vs 28.7) in both the intent-to-treat and per-protocol efficacy analysis datasets ($P = 0.0003$ and $P = 0.006$, respectively). In addition, the frequency of new target joint bleeds was significantly lower in the prophylactic arm (7 new joint bleeds in 5 of 17 patients), when compared with their frequency in the on-demand arm (23 new joint bleeds in 11 of 19 patients; $P = 0.027$).

A total of 104 adverse events were reported, including 30 serious and 74 nonserious events. In all, 27 events (26%) were believed to be related to FEIBA NF therapy, including one non-serious hypersensitivity reaction. No thromboembolic events occurred. At termination of the study, seven previously negative patients tested positive for hepatitis B surface antibody (HBsAb), although all were negative for hepatitis B core antibody, hepatitis

**TABLE 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Prophylaxis (n = 17)</th>
<th>On-demand (n = 19)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding episodes</td>
<td>196</td>
<td>629</td>
<td></td>
</tr>
<tr>
<td>Annualized bleeding rate (ABR), median</td>
<td>7.9</td>
<td>28.7</td>
<td></td>
</tr>
<tr>
<td>Occurrence of new target joints</td>
<td>7 in 5/17 patients</td>
<td>23 in 11/19 patients</td>
<td></td>
</tr>
<tr>
<td>New target joint ABR, median</td>
<td>0</td>
<td>5.9</td>
<td>0.027</td>
</tr>
</tbody>
</table>

FEIBA NF = Factor Eight Inhibitor Bypass Activity Nanofiltered

* Patients (median age, 23 years) with hemophilia A or B had inhibitors against factor replacement concentrates that were refractory to factor VIII or IX.

* Received 85 ± 15 U/kg of FEIBA NF every other day for 12 months ± 14 days

* Received FEIBA NF for control of acute bleeding at dosages determined by the treating physicians

Source: Antunes et al

Holleh D. Husseinzadeh, MD  Expanding Therapeutic Options for Hemophilia A and B: Results of Recent Trials and Research
B surface antigen, and hepatitis B virus DNA. These findings seemed to result from passive transmission of HBsAb from the donor blood product. One patient in the prophylaxis arm and two patients in the on-demand arm experienced a rise in inhibitor level.

Thus, prophylactic, recurrent administration of FEIBA NF appeared in this study to be as safe as on-demand therapy while significantly reducing all types of bleeds by 72.5% in patients with hemophilia A or B who had developed inhibitors to factor replacement concentrates. Two of the patients who received FEIBA NF prophylactically for 12 months had no bleeding episodes.

**Patient Adherence to Factor Infusion Therapy**

The relationship between compliance with factor infusion therapy and specific health outcomes is not well documented. Vietri and colleagues assessed the relationship between adherence to prophylactic factor infusions in adults and children with moderate-to-severe hemophilia A or B and outcomes, including bleeding events and patient-reported health status.

Adult hemophilia patients or parents of minors with hemophilia were identified through databases at hemophilia treatment centers. A total of 53 adults with moderate or severe hemophilia A (n = 43) or B (n = 10) and 56 children with hemophilia receiving prophylaxis participated in the study. Study participants completed online questionnaires, which included an adherence assessment, using the Validated Hemophilia Regimen Treatment Adherence Scale–Prophylaxis (VERITAS-Pro), and a self-reported evaluation of health status, using the 12-item Short-Form Health Survey (SF-12v2) for adults and the SF-10 for children. Study participants completed online questionnaires, which included an adherence assessment, using the Validated Hemophilia Regimen Treatment Adherence Scale–Prophylaxis (VERITAS-Pro), and a self-reported evaluation of health status, using the 12-item Short-Form Health Survey (SF-12v2) for adults and the SF-10 for children. Participants in the study were asked to provide the numbers of breakthrough bleeding events, emergency department visits, hospital admissions, and missed days of work or school they or their children experienced due to bleeding episodes.

In adult patients with hemophilia A or B, lower adherence was associated with an increased number of bleeding episodes requiring administration of replacement factors (P < 0.001), as well as more days of work or school missed in the past year because of bleeding episodes (P < 0.05). No significant association between adherence and self-reported health status was found among adults (P = 0.91), but increased adherence in children was associated with better physical health (P < 0.01). In pediatric patients, adherence was not significantly associated with the reported number of bleeding episodes (P = 0.95), but it was significantly associated with infection at the injection site (P < 0.05), increased hospital stays for bleeding episodes (P < 0.001), and missed work/school days due to bleeding episodes (P < 0.01).

Although the sample size was limited, these results showed that greater adherence to a prophylactic factor replacement regimen by both adults and children with moderate-to-severe hemophilia A or B was associated with significantly better clinical outcomes.

**Improving Outcomes After Total Joint Replacement**

Goto et al presented a retrospective review of subjective and objective outcomes in patients with congenital bleeding disorders after they received total joint arthroplasty (TJA) at a single institution. The goals were assessment of outcomes of TJA for hemophilic arthropathy and the safety and efficacy of standard pharmacologic thromboprophylaxis in this population.

A retrospective chart review of 28 patients with various congenital bleeding disorders, including hemophilia A (n = 21), hemophilia B (n = 4), factor-11 deficiency (n = 1), and von Willebrand disease (n = 2), yielded outcomes data from 38 arthroplastic procedures, including 29 instances of total knee arthroplasty (TKA) and 9 cases of total hip arthroplasty (THA).

Outcomes are summarized in Table 2. Objective postoperative clinical outcome data at 2 months were available for 27 of the 28 patients. Seven of the 28 patients (25%) had improvement in range of motion (median, 15°; range, 5°–25°). At 1.5 years postoperatively, 17 of 29 TKA patients (59%) experienced improved range of motion (median, 15°; range, 15°–45°), and 100% had decreased knee pain. Two months postoperatively, all nine THA patients had improved range of motion, including internal rotation (89%; median, 45°), external rotation (100%; median, 30°), flexion (56%; median, 35°), extension (78%; median, 15°), and abduction (78%; median, 15°).

Twenty-two of the 28 study patients could be contacted for subjective responses to arthroplasty. All 25 patients undergoing TKA reported significant improvement in pain; 24 of the 25 patients (96%) reported improvement in joint function after joint replacement, and all 25 would elect to repeat the surgery if asked to make the choice again. All six THA patients reported improved joint pain and function, and five of the six (83%) stated they would choose to have surgery if presented with the option again.

Low-molecular-weight heparin (LMWH) was used as thromboprophylaxis in 29 of 38 procedures (76%), but it was discontinued in three patients for non-joint bleeding or suspected blood loss (two cases of hypotension and anemia). No symptomatic venothrombotic events occurred. Early complications of TJA included cellulitis in five patients and hemarthrosis in two patients not on LMWH prophylaxis. Observed late complications of surgery included aseptic loosening of knee arthroplasties requiring repeated TKA, one case of septic arthritis, and development of severe flexion contracture requiring repeated TKA.

**Linking Obesity with Decreased Compliance**

Home infusion of clotting factors is important for proper prophylaxis and treatment of acute bleeding episodes in hemophilia. To be effective, patients need to have both an adequate supply of factor as well as the skill and ability to establish access for intravenous administration. This retrospective analysis, presented by Ullman and others, investigated whether an elevated body mass index (BMI) was associated with decreased use of factor home-infusion treatment (HI) and self-
infusion (SI) among patients with hemophilia A or B in the United States. The investigators analyzed data from 10,814 males aged 6–79 years old. Patients with human immunodeficiency virus (HIV) infection, symptomatic liver disease, or an inhibitor titer > 5 Bethesda units were excluded from the analysis. The prevalence of HI and SI was recorded with demographic and clinical characteristics. Bivariate relationships were assessed using the χ² test, and independent associations between BMI and HI/SI were evaluated by logistic regression.

Paralleling current trends in the US population, 50% of analyzed males in the study cohort were overweight or obese. In all, 70% of patients studied used HI; 44% of those also used SI. Overweight and obese individuals were less likely to use HI than were those of normal weight (odds ratio [OR] = 0.8; 95% confidence interval [CI] = 0.7–1.0 and OR = 0.7; 95% CI = 0.6–0.8, respectively.) Obese teens and adults also were less likely to participate in HI than were those of normal weight (OR = 0.8; 95% CI = 0.7–0.9). Among all patients, SI use declined after age 40 years, no matter what the patient’s severity of disease or prophylactic treatment regimen used.

These results suggest that overweight and obese persons with hemophilia A or B may be less likely to use HI or SI—perhaps because of the increased difficulty of performing venipuncture due to excess adiposity—resulting in irregularity of prophylaxis, delayed treatment of bleeding episodes, and increased risk of hemophilia-related complications.

Arteriovenous Fistulae (AVF) in Patients with Bleeding Disorders

Venous access is a major issue in facilitating factor administration in patients with bleeding disorders. Tapia and others reported on long-term follow-up of 17 such patients, including 2 patients with von Willebrand disease, 12 with hemophilia A (3 with inhibitors), and 3 with hemophilia B (1 with an inhibitor) who had AVF inserted for venous access. At a mean follow-up of 5 years (range, 1–15 years), 15 patients reported “excellent” results, with continued fistula viability. No patients had bleeding complications, AVF-related infection, or difficulty achieving venous access for administration of factor replacements. Four patients (24%), however, reported dissatisfaction with the appearance of the AVF, resulting in one revision procedure with improvement of appearance.

AVF are a viable option associated with overall satisfaction in patients with bleeding disorders who require long-term venous access. This procedure may be considered in individuals with difficult access or those who have had repeated complications with existing modes of access. However, AVF are associated with a measurable rate of dissatisfaction in cosmetic appearance.

Determining Food Insecurity in Hemophilia Patients

Zia et al sought to identify the prevalence of food insecurity in families of hemophilia patients at a single treatment center. Patients were screened during annual comprehensive visits from May 2012 to January 2013 using a two-question validated screening tool along with their general health assessments.

Data from 42 male children (age, 0–18 years) were analyzed. The overall prevalence of food insecurity was 16.7%, which was similar to that of the national averages. Food insecurity was less prevalent among those with mild or moderate disease (5.6%) than among those with severe hemophilia (25.0%; 95% CI = 7.7–42.3). Children who tended to be at increased risk of food insecurity were older, taller, or heavier than the other children; had a higher BMI; or belonged to a minority; however, none of these characteristics had a significant impact (P > 0.05) on food insecurity in this small sample population. Nevertheless, these results highlight the need for food-insecurity screening among hemophilia patients and their families and connection of these families to appropriate community resources.

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Treatment of Hemophilia: What’s in the Pipeline?
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Indiana University School of Medicine, Indianapolis, Indiana

Abstract  Current management of hemophilia is based on factor-replacement therapy. Because plasma-derived and recombinant factor products have short half-lives, they demand frequent dosing, which, in turn, leads to issues with venous access, inhibitor development, and high financial cost. Bioengineering strategies are bringing new and promising products down the pipeline that may improve patient outcomes via more effective bleeding control and preservation of joint function; reduce the burden of administration by reducing dosing frequency and providing more cost-effective therapy; individualize treatment by adapting to individual pharmacokinetics and personalizing treatment regimens based on variability of bleeding phenotype and lifestyle; identify, monitor, and prevent age-related comorbidities; and potentially develop a cure for hemophilia through gene therapy.

Hemophilia A and B are hereditary, X chromosome-linked, recessive bleeding disorders caused by mutations in genes for factor VIII (FVIII; F8) and factor IX (FIX; F9). Both factors are essential components in the coagulation pathway. The prevalence of hemophilia commonly is reported as 1:5,000 live male births for hemophilia A and 1:30,000 for hemophilia B. Hemophilia classification is based on factor activity levels and range from mild (6%–40% activity) and moderate (1%–5% activity) to severe (< 1% activity). The bleeding phenotype generally correlates with the level of factor deficiency. Patients with severe hemophilia experience bleeding episodes involving joints, soft tissues, and muscles. Repetitive bleeding into joints can lead to debilitating chronic arthropathy with limited joint mobility, flexion contracture, and muscle wasting.1-5

This report on the present and future of hemophilia management is based on a presentation by Steven W. Pipe, MD, Professor of Pediatrics and Director of the Division of Pediatric Hematology/Oncology at the University of Michigan Health System in Ann Arbor. Dr. Pipe spoke during a symposium sponsored by Biogen Idec at the 65th Annual Meeting of the National Hemophilia Foundation in Anaheim, California.

CURRENT THERAPY OF HEMOPHILIA
Current therapy of hemophilia is based on the replacement of missing clotting factors. Factor is derived from either donated human plasma or the more recent and favored recombinant DNA technology.5 Prophylactic factor administration, the current standard of care for severe hemophilia, is started early in life, preferably before the onset of hemarthroses.5 Prophylactic FVIII commonly is given at a dosage of 25–50 IU/kg every other day or 3 days/week, assuming an average recovery of 2 IU/dL for each IU/kg infused. Prophylactic FIX commonly is given at a dosage of 25–40 IU/kg twice weekly, assuming an average recovery of 1 IU/dL for each IU/kg infused.4 Current factor products have limitations. First, the short half-lives of 8–12 hours for FVIII and 18–24 hours for FIX require frequent dosing in prophylactic settings and repeat dosing in on-demand settings.4 Second, the development of neutralizing antibodies (inhibitors) renders factor replacement ineffective; these inhibitors develop in up to 30% of patients with hemophilia A and 3%–5% of patients with hemophilia B. Third, current therapies require venous access, which may necessitate the placement of central venous access devices in young children. These devices carry the risk for infection and thrombosis.5 Fourth, the cost of current therapies limits their availability to many patients with hemophilia worldwide.6

Results of an investigation of healthcare resource use by Valentino et al7 showed that the median annual hemophilia A–related costs from 2001 to 2007 in the United States were $63,935. This cost increased significantly to a median of $271,357 annually in patients who developed inhibitors. The median annual cost increased significantly from birth to about 15 years of age; thereafter, costs remained stable before dropping again at 21 years of age. The current annual cost of prophylactic factor use is over $150,000.4 Because of this, it is estimated that two thirds of the world do not have proper access to replacement therapies.5,6
GOALS OF THE HEMOPHILIA THERAPY PIPELINE

The past four decades have witnessed great advances in hemophilia therapy (Table 1). Goals include improving patient outcomes with more effective bleeding control and preservation of joint function; reducing the burden of factor administration through decreases in dosing frequency and more cost-effective therapy; individualizing therapy by adapting to individual pharmacokinetics and personalizing treatment regimens based on the variability of bleeding phenotype and lifestyle; identifying, monitoring, and preventing age-related comorbidities; and developing a cure for hemophilia through gene therapy. Table 2 provides a list of current clinical trials of novel recombinant factors in hemophilia A and B.

EXTENDING THE HALF-LIFE OF RECOMBINANT FACTOR

Current factor research has focused on the development of longer-acting products to decrease the frequency and dosing of factor infusions, improve compliance with prophylactic regimens, prevent bleeding episodes and re-bleeding with bleeding episodes, and improve the overall quality of life for patients with hemophilia. Investigations into extending the half-life of current recombinant coagulation factors have used several strategies, including reduction of exposure to clearance receptors through PEGylation, rescue of endocytosed proteins from intracellular degradation by crystallizable fragment (Fc) fusion and albumin fusion proteins, and enhanced interactions with von Willebrand factor (vWF).

PEGylation

PEGylation improves drug efficacy via the covalent attachment of polyethylene glycol molecules (PEG) to the protein of interest—in this case, recombinant factor proteins (Figure 1). The PEG structures attract water molecules that surround factor proteins. For many therapeutic proteins employing PEGylation, this process effectively increases the size of the protein beyond renal filtration ability. Because factor proteins are already too large for kidney filtration, PEGylation provides other benefits, possibly through disrupted interactions with clearance receptors, and may decrease interactions with immune-mediating cells.

PEGylation of therapeutic proteins, which has been in clinical use since 1990, is considered to be safe and well tolerated. Early PEGylation of factor proteins used random introduction of PEG molecules, which often interfered with factor ability to function with reduced coagulant activity and disrupted vWF-binding in FVIII. This led to targeted, site-specific PEGylation of factor proteins to preserve functional protein-protein interactions.

Factor VIII. A phase 1 study of site-specific PEGylated recombinant FVIII (rFVIII, BAY 94-9027) demonstrated improved pharmacokinetics with a terminal half-life of 19 hours (twice that of B-domain deleted rFVIII). BAY 94-9027 was well tolerated; no adverse events were reported, and no inhibitors developed in patients who received it. A phase 2/3 study is ongoing.

A phase 1 study of a similar PEGylated rFVIII, N8-GP replicated these results, with a terminal half-life of 19 hours, no adverse events, and no inhibitor development. Testing of a third site-specific PEGylated rFVIII, BAX 855, demonstrated promising preclinical pharmacokinetic...
ics with, again, a twofold prolongation of the terminal half-life of rFVIII.12 A phase 1 trial recently was completed, and a phase 2/3 trial is ongoing.

**Factor IX.** In a phase 1 study of a site-specific PEGylated recombinant FIX (rFIX) known as N9-GP, 16 previously treated patients received one dose of their usual factor replacement product followed by the same single dose of N9-GP. This trial revealed an astounding difference in pharmacokinetics, with an N9-GP terminal half-life of 93 hours (fivefold longer than the half-life of rFIX). In contrast to the excellent patient tolerability of PEGylated rFVIII, however, 1 of the 16 patients treated with N9-GP developed a transient hypersensitivity to it and withdrew from the trial. None of the patients receiving N9-GP developed inhibitors.13

A population-based pharmacokinetic model has been created based on the N9-GP phase 1 data. Simulated N9-GP dosing levels have suggested the possibility of prophylactic dosing by administering a single dose every 2 weeks. Simulations for on-demand therapy predicted FIX levels above 40 IU/dL for an average of 23 hours with one 40 U/kg dose, which replaced two consecutive doses of standard FIX concentrate given every 12 hours.14 Phase 3 trials in both adults and children are ongoing.

**PEGylated Liposomes**

Another approach to prolonging the half-life of recombinant factor proteins is by attaching them to the outer surface of PEGylated liposomes via noncovalent binding (Figure 2).4 The liposomes encapsulate the factor proteins and serve as carriers, interfering with the ability of the reticuloendothelial system to recognize the factor, thereby prolonging its half-life.4

**Factor VIII.** In a phase 1 study, 12 patients were treated with two dose levels of PEGylated liposomes (rFVIII PEG-Lip; BAY 79-4980). Researchers noted better tolerability and longer bleed-free intervals with the use of rFVIII PEG-Lip than with administration of standard rFVIII.15 However, subsequent pharmacokinetic studies showed that rFVIII PEG-Lip offered no benefit over standard rFVIII.4,5 Phase 2 studies were terminated early for endpoint failure.

**Polysialylation**

Polysialic acids (PSAs) can serve as an alternative to PEGylation (Figure 3).1 When these N-acetylneuraminic acid polymers are attached to recombinant factor proteins, they attract water and produce a watery “cloud” that surrounds

### TABLE 2

Current Clinical Trials of Novel Recombinant Factors in Hemophilia A and B

<table>
<thead>
<tr>
<th>Factor</th>
<th>Modification</th>
<th>Product</th>
<th>Manufacturer</th>
<th>Current status</th>
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<tr>
<td>FVIII</td>
<td>New recombinant</td>
<td>Octagene</td>
<td>Octapharma</td>
<td>Phase 3 trial completed; trial in previously untreated patients ongoing</td>
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<td>Bayer</td>
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<td>rFVIII PEG-Lip</td>
<td>BAY 79-4980</td>
<td>Bayer</td>
<td>Phase 1 trial completed; phase 2 trial terminated for endpoint failure</td>
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<tr>
<td>Site-specific PEG rFVIII</td>
<td>BAY 94-9027</td>
<td>Bayer</td>
<td>Phase 1 trial completed; phase 2/3 trials ongoing</td>
<td></td>
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<tr>
<td>Site-specific PEG rFVIII</td>
<td>NB-GP</td>
<td>Novo Nordisk</td>
<td>Phase 1 trial completed; phase 3 trial ongoing</td>
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<tr>
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<td>Baxter</td>
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<td>CSL Behring</td>
<td>Phase 2/3 trials ongoing</td>
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<td>IB1001</td>
<td>Cangene</td>
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<td>BAX 326</td>
<td>Baxter</td>
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<td>Phase 1/2/3 trials completed; pediatric phase 3 trial ongoing</td>
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<td>hBS23</td>
<td>Chugai</td>
<td>Phase 1 trial ongoing in Japan</td>
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</tbody>
</table>

FVIII = factor VIII; rFVIII = recombinant factor VIII; PEG = PEGylated; FIX = factor IX; rFIX = recombinant factor IX; FVII = factor VII; rFVIIa or rhFVIIa = recombinant activated factor VII. 
Source: Peyvandi et al,4 Pipe,9 ClinicalTrials.gov website18

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![FIGURE 2 Schematic representation of PEGylated liposomes. PEG = polyethylene glycol. Reproduced, with permission, from Peyvandi et al.4](Image)
the protein to protect it from clearance receptors, proteolytic enzymes, and immune-mediating cells.5,8

Unlike PEG, PSAs are naturally occurring and biodegradable.3 Polysialylation of rFVIII, FIX, activated factor FVII (FVIIa), and vWF are all currently under study.

**Fc Fusion**

Fusion protein technology links factor to another naturally occurring protein with a much longer half-life, such as immunoglobulin G (IgG; Figure 3).4 Fusion of factor to the Fc portion of IgG protects the factor protein from lysosomal degradation via interactions with neonatal Fc receptors when internalized by endothelial cells. Phagocytized fusion proteins are recycled back to the cell surface in a pH-dependent manner and re-released into plasma.5,16,17

**Factor VIII.** A phase 1/2, first-in-human trial of rFVIII-Fc involved 16 patients who received a single dose of rFVIII followed by an equal dose of rFVIII-Fc. All doses were well tolerated, no adverse events related to study drug were reported, and none of the patients developed inhibitors. Pharmacokinetic studies demonstrated a terminal half-life 1.6-fold longer than that of rFVIII; clearance was 1.5-fold lower.16

A follow-up phase 3 trial in adults was completed recently. Early results demonstrated tolerability in 165 patients, with no reported adverse events or inhibitor development. Pharmacokinetic studies demonstrated a terminal half-life of 19 hours, with a mean interval between doses of 3.5 days to maintain prophylactic factor activity levels; 30% of the patients in the active treatment arm were dosed every 5 days. For on-demand dosing, 87% of bleeding episodes were controlled with one dose of rFVIII-Fc, and 98% of the the patients achieved bleeding control with one or two doses.18 A phase 3 trial in children is ongoing.

**Factor IX.** In a phase 1/2, dose-escalation trial of rFIX-Fc, 14 patients demonstrated a terminal half-life of 56.7 hours (approximately threefold longer than the half-life of standard rFIX products). rFIX-Fc was well tolerated; no serious adverse events or inhibitor development was reported.19 Pharmacokinetic modeling based on the results of this study suggested that dosing once every 2 weeks at 100 IU/kg was sufficient for trough levels 1% above baseline.19

A subsequent phase 3 study in adults was completed recently. Early results demonstrated overall tolerability in 123 patients; one episode of obstructive uropathy was reported. None of the patients developed inhibitors. Pharmacokinetic studies demonstrated a remarkable terminal half-life of 82 hours (two- to threefold longer than with rFIX), suggesting that prophylactic dosing could be given once every 2 weeks. Further, 54% of patients given individualized prophylactic dosing achieved dosing intervals ≥14 days. Although 90% of bleeding episodes were controlled with a single on-demand dose of rFIX-Fc, 97% of bleeding episodes were controlled with one or two doses.20 A phase 3 study in children is ongoing.

**Albumin Fusion**

Similar to Fc fusion, albumin fusion technology links factor proteins with human albumin, a natural carrier molecule (Figure 5).4 The resulting albumin-bound molecule has a longer half-life than that of currently available coagulation factors, protects the factor protein from proteolytic degradation, and may prevent exposure to immune-mediating cells, thus prolonging its half-life and decreasing immunogenicity via the same recycling mechanism as described for Fc fusion.4,8

**Factor IX.** Results of a phase 1, first-in-human, dose-escalation study of the fusion protein linking rFIX with albumin (rFIX-FP) are promising. In 25 patients, rFIX-FP demonstrated a favorable safety profile and a remarkable pharmacokinetic profile, offering a half-life fivefold longer than that associated with other FIX products.21 The follow-up phase 1/2 study of rFIX-FP echoed these promising results. Preliminary data demonstrated a pharmacokinetic profile suggesting that weekly prophylactic dosing with rFIX-FP provides bleeding prophylaxis and that extended dosing intervals of 10–14 days may be feasible.22 Phase 3 trials in adults and children are ongoing.

**Factor VIIIa.** In a first-in-human study of rFVIIa-FP, 40 healthy subjects were dosed in five consecutive groups. All doses were well tolerated, no serious adverse events were reported, and none of the subjects developed inhibitors. Pharmacokinetic data revealed a mean terminal half-life of 8.5 hours (about 3.5-fold longer than that of commercial rFVIIa products).23
naturally protects it from degradation. A unique recombinant, single-chain rFVIII (CSL627) has shown improved stability and higher affinity for vWF when compared with other rFVIII formulations.24

In preclinical studies, CSL627 has demonstrated safety and efficacy with equivalent hemostatic activity as compared with full-length rFVIII formulations. The novel single-chain design provides for higher intrinsic stability and affinity for vWF.25 Whether this translates into a reduced immunogenic potential will be investigated in a recently commenced phase 1/3 clinical trial.

**Bispecific Antibody**

In addition to improving factor proteins themselves, a novel approach has been taken to replace FVIII cofactor function by a small molecule.26 This humanized bispecific antibody to FIXa and FX (hBS23) binds FIXa with one arm and FX with the other.

Figure 6 illustrates a bispecific antibody to mimic FVIIIa activity.27 This molecule brings the two factors together into appropriate positions for hemostatic activity. The most promising hBS23 preclinical study revealed a terminal half-life of 14 days and subcutaneous bioavailability of nearly 100%.27

## GENE THERAPY

Gene therapy involves the transfer of a normal copy of a gene into a person who harbors a mutation of that gene. Despite early disappointments of gene therapy in hemophilia,28 recent studies of new gene-transfer technology have renewed interest in this potentially curative approach.

Gene therapy trials have used plasmids, retroviral, and adenoviral vectors directed to autologous fibroblasts, hematopoietic stem cells, and target cells (eg, skeletal muscle and liver cells). However, investigators have been hampered by low levels of gene expression and failure to achieve long-term gene expression.24 With newer adeno-associated viral-mediated (AAV) models, gene therapy is again under investigation.29 Hemophilia is a good candidate for gene therapy, because it is a monogenetic disease that requires the production of only a small fraction of normal factor activity to ameliorate or cure the bleeding phenotype.

Using AAV-8 delivery of an F9 transgene, researchers at University College London documented post–gene transfer FIX levels of 5%–10% and stable FIX levels extending beyond 30 weeks.30 The biggest challenge of gene-transfer therapy is modulating host immune responses to the vector and gene product. Patients in this study experienced mild transaminits, which likely resulted from a host immune response to the adenoviral vector. This response apparently was successfully managed with a short course of corticosteroids. No adverse effect on gene transfer or resulting plasma factor levels was noted.30 Thus, gene therapy apparently has achieved long-term expression of therapeutic FIX levels,31 but successes with FVIII are lagging behind.

**Using Genetic Variants Instead of Normal Genes**

Perhaps the two most interesting advancements in gene therapy include the use of lentiviral vectors and the manipulation of genetic variants to produce overexpression of factor as a transgene. Lentiviral vectors can incorporate larger transgenes, which may facilitate FVIII transfer. Lentiviral vectors also avoid pre-existing immunity, which can be a barrier to the use of AAV vectors.31

The idea of using an F9 genetic variant to achieve overexpression of FIX instead of using the normal F9 gene to achieve normal expression recently was investigated.32 Preclinical mouse models using AAV-8 delivery of a genetically modified F9 transgene resulted in over 55% FIX activity. This promising work has resulted in a current human trial (ASKBIO009), which has just enrolled its first two patients.

## AGING AND COMORBIDITIES

With improved availability of quality factor concentrates for factor replacement, implementation of prophylactic regimens and advancements in screening, and treatment of blood-borne viral infections, people with hemophilia are now living longer than ever before.1 Improved life expectancy comes with the typical comorbidities seen in an aging population, such as cardiovascular and renal diseases and various malignancies.33–35 These issues are being explored, and general aspects of clinical management have been suggested.36–38 These issues will become more prevalent as the hemophilia population ages and will require further attention.

## CONCLUSION

Potential therapies in the pipeline to treat hemophilia focus on prolonging the half-life of recombinant factor products, improving patient outcomes, and developing a cure for hemophilia through gene therapy. Analysis of available data suggests that these early trials appear to be meeting their goals.

At least three different FIX products of three different engineering methods

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**FIGURE 6** A bispecific antibody capable of mimicking activated factor VIII (FVIIIa) activity. FIXa = activated factor IX; FX = factor X; HC = heavy chain; LC = light chain. Reproduced, with permission, from Kitazawa et al.27
have shown terminal half-lives as much as fivefold greater than those of standard rFIX products. Prolonging the time that plasma levels can be measured during prophylactic factor use translates into a need for fewer factor infusions and dosing intervals lasting as long as 2 weeks. Available data on newly engineered FVIII products have been somewhat less promising. These products currently have terminal half-lives of up to 1.8 times greater than those of standard rFVIII factor. Ultimately, ongoing phase 3 trials will establish the effect this technology will have on individual dosing schedules, overall factor use, patient compliance, inhibitor development, need for venous access, and overall cost of therapy.

Gene therapy is equally as promising, and a new wave of human trials is ongoing. The most recent trials have achieved long-term expression of therapeutic factor levels. The field is exploring ways to improve gene delivery, minimize vector immunogenicity, prolong gene expression, and raise factor activity levels.

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Recent Advances in Preventing Bleeding, Reducing Inhibitors, and Managing Acute Bleeding

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Abstract Recent advances in the prevention of bleeding episodes, reduction of inhibitory alloantibodies to factor concentrates (inhibitors), and management of acute bleeding episodes in hemophilia were presented at the 55th Annual Meeting of the American Society of Hematology (ASH). Among the more notable observations were: (1) bleeding tendency is associated with the level of factor VIII (FVIII) activity in patients receiving prophylactic recombinant FVIII crystallizable fragment (Fc) fusion protein; (2) prophylactic treatment with long-lasting recombinant factor IX Fc fusion protein is safe and effective in adolescents with hemophilia B; (3) delayed prophylaxis has long-term deleterious effects in patients with acute hemophilia; (4) magnetic resonance imaging is useful for detecting early hemophilic joint disease; (5) early, low-dose prophylaxis in the absence of immunologic danger signals may reduce inhibitor incidence in patients with hemophilia A; (6) prompt immune tolerance induction at inhibitor diagnosis may increase the success rate in patients with hemophilia A and inhibitors; and (7) four-factor prothrombin complex is superior to plasma administration for achieving hemostasis before urgent surgery. Ongoing studies on prophylaxis, reduction and management of inhibitors, and treatment of acute bleeding are crucial to continuing improvement in the quality of life of patients with hemophilia.

Hemophilia is caused by a functional or quantitative deficiency of a coagulation protein—factor VIII (FVIII) in hemophilia A and factor IX (FIX) in hemophilia B—and can lead to a lifelong bleeding disorder. Uncontrolled or unrecognized major bleeding events may result in joint damage, intracranial hemorrhage, and death. Complications of hemophilia have included musculoskeletal disease, development of inhibitory antibodies, and transmission of viral bloodborne infections. Before the advent of efficient coagulation factor replacement therapy, the life expectancy of a child with severe hemophilia A was about 20 years. Currently, the life expectancy of this population approximates that of the general population.

Approval of the first recombinant factor VIII (rFVIII) concentrates in the United States in 1992 improved the product supply, decreased the incidence of viral bloodborne infections to near zero, and advanced the adoption of prophylactic treatment regimens. Prophylactic treatment regimens achieve a less severe clinical phenotype by maintaining hemostatically protective factor levels, accomplished with regularly scheduled infusions. Specific regimens to achieve optimal bleed suppression and prevention vary. Current standard prophylactic regimens commonly use infusion therapy given three times weekly, whereas other regimens require administration every other day. Regimens may be individually modified to achieve optimal bleed suppression and tailored to the patient’s individual needs. Often, patients have difficulty in adhering to demanding therapeutic prophylactic regimens that include frequent morning infusion to achieve adequate hemostatic coverage during periods of highest activity. As children progress to adolescence, adherence is often further limited, making these regimens less effective.

Inhibitors, or inhibitory alloantibodies to factor concentrates, develop in approximately 25%–30% of patients with severe hemophilia A and 3%–13% of those with moderate or mild disease. Inhibitors neutralize infused FVIII and greatly affect a patient’s ability to achieve hemostasis. A bleeding episode in patients with high inhibitor titers requires the use of a bypassing agent; effective use of prophylaxis is greatly diminished in this subset of patients. Patients with inhibitors require considerable management expertise. The use of bypassing agents and immunotolerance regimens that aim to decrease the inhibitory alloantibodies remain enormously costly. Therefore, much work remains in determining predictors of inhibitor development and in developing regimens to modify the individual’s immune response.

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This report will discuss recent advances in prophylaxis and imaging to prevent bleeding episodes, reduction of the incidence of inhibitors, and management of acute bleeding episodes in hemophilia patients.

PREVENTION OF BLEEDING EPISODES: USE OF PROPHYLAXIS AND IMAGING

Bleeding Tendency and FVIII Activity Level

Joints can be preserved and bleeding can be minimized when circulating FVIII levels remain ≥ 1 IU/dL. Patients receiving prophylaxis with recombinant FVIII who have had prolonged intervals of circulating FVIII levels < 1 IU/dL experience an increase in bleeding episodes and hemarthroses. Thus, prophylactic regimens used in patients with hemophilia A have been designed to maintain the FVIII activity ≥ 1 IU/dL.

Pasi et al. examined bleeding tendency in relation to predicted FVIII activity levels in patients with severe hemophilia A who were treated with recombinant FVIII crystallizable fragment (Fc) fusion protein (rFVIIIFc) in the A-LONG study. A two-compartment population pharmacokinetic model of rFVIIIFc was developed based on activity-time profiles in 180 patients with severe hemophilia A who were 12–65 years of age. Negative binomial regression models were used to correlate the number of bleeding episodes with the annualized time (in days) that the patients’ FVIII activity levels were < 1 IU/dL. Models were adjusted for age, body mass index, baseline human immunodeficiency virus (HIV) and hepatitis C virus status, baseline von Willebrand factor level, number of bleeding episodes, and time on study.

Multivariable negative binomial regression analysis showed that the number of overall bleeding episodes increased the more time that patients experienced a FVIII activity level < 1 IU/dL (P < 0.001). A significant association also was found between the length of time in which patients had a FVIII activity level < 1 IU/dL and different types of bleeding (spontaneous, traumatic, or joint). In addition to their increased bleeding tendency, the chance that these patients would be bleeding-free was lessened. These findings reinforce the importance of a therapeutic threshold of 1 IU/dL of FVIII activity and contribute to building a stronger foundation for designing effective rFVIIIIFc prophylactic dose regimens.

Long-Lasting Recombinant Factor IX Fc Fusion Protein (rFIXFc)

Prophylactic replacement of FIX, which is used to treat people with hemophilia B, requires up to three weekly intravenous injections. To reduce the frequency of injections, a long-acting recombinant protein (rFIXFc) consisting of one rFIX molecule covalently bonded to the Fc domain of immunoglobulin G1 (IgG1) was developed. Fc fusion uses an endogenous IgG recycling pathway to prolong the half-life of therapeutic proteins. The phase 3 B-LONG study (ClinicalTrials.gov ID No. NCT01027364) examined the pharmacokinetics, safety, and efficacy of rFIXFc given to previously treated subjects with hemophilia B. Age may affect the half-life of recombinant coagulation factors.

A planned subgroup analysis by Shapiro et al. examined whether age influenced the pharmacokinetics, safety, and efficacy of rFIXFc use. Male subjects with severe hemophilia B (≤ 2 IU/dL) who were at least 12 years of age, who were without inhibitors to FIX, and who had ≥ 100 exposure days to FIX were assigned to one of four treatment arms. Arm 1 included those who had weekly prophylaxis, arm 2 included those who had individualized interval prophylaxis, arm 3 included those who had episodic treatment of bleeding episodes, and arm 4 included those who had perioperative management. The study was stopped when prespecified exposure was achieved and the specified number of major surgical procedures were completed. The pharmacokinetic parameters of rFIXFc were estimated in all study participants by a noncompartmental analysis based on a one-stage clotting assay. A sequential subgroup analysis compared the pharmacokinetic profile of rFIXFc with that of rFIX in arm 1. The primary endpoints were annualized bleeding rate (ABR), the incidence of adverse events, and inhibitor development. This subgroup analysis compared the outcomes in adolescents (12–17 years of age) with those of adults (18–65 years of age). Efficacy of rFIXFc was assessed by comparing the ABR in arms 1 and 2 with that in arm 3 separately for adolescents and adults.

A total of 123 patients were enrolled at 50 study centers in 17 countries. The median treatment duration was 51.4 weeks across all arms. Eleven adolescents were enrolled in arms 1–3 and none in the perioperative management arm (arm 4). All adolescent patients had evaluable rFIXFc pharmacokinetic profiles. The terminal half-life, clearance, incremental recovery, and volume of distribution at steady state were similar between adolescents and adults. The ABRs of adolescents also were comparable to those of adults. Both adolescent and adult subgroups in the prophylactic arms (arms 1 and 2) had lower ABRs than those in the episodic treatment arm (arm 3). No distinctive safety issues were observed in adolescents; their safety profile was similar to that of the adult population.

Pharmacokinetic parameters, safety profiles, and ABRs with rFIXFc prophylaxis were similar in adults and adolescents. This analysis supported the primary result of the B-LONG study, showing that rFIXFc is well tolerated and effective for preventing bleeding episodes in both adults and adolescents with hemophilia B.

Delayed Prophylaxis Has Long-Term Effects in Patients with Severe Hemophilia

The Joint Outcome Study (JOS) was a randomized, controlled clinical trial in boys with severe FVIII deficiency comparing prophylaxis (ie, 25 IU/kg of FVIII given every other day, beginning before age 30 months) with an enhanced episodic regimen used only in response to bleeding. At age 6, patients were discontinued from the study, and joint outcome by sensitive magnetic resonance imaging (MRI) and physical examination of six index joints (including both ankles, knees, and elbows) were evaluated. The JOS data...
demonstrated the superiority of prophylaxis over episodic therapy (P < 0.05). At the end of the study, all parents were informed of the study results, and patients were switched to prophylaxis.

The JOS Continuation Study (JOSc) currently is being performed to determine the results of early prophylaxis on joint development until age 18 years and the impact of delaying initiation of prophylaxis until age 6 years. All boys in the original JOS trial were eligible to enroll in the continuation study. Data being collected include the cumulative number of index, joint, and total hemorrhages; joint physical examination score of six index joints, using the Colorado Pediatric Joint Assessment Scale;18 MRI, soft-tissue, osteochondral, and total scores; and total scores of six index joints using the expanded MRI 45 Scale. Additional data are being collected on prophylaxis adherence, activities, surgeries, quality of life, and replacement factor use.

To date, results from 26 (40%) of the original 65 boys, including 16 on early and 10 on delayed prophylaxis, comprising a total of 156 index joints are available for analysis. Following delayed initiation of prophylaxis, adolescents manifested increased numbers of hemarthroses and increased MRI damage affecting particularly their bones and cartilage. MRI was more sensitive than was joint physical examination in determining joint outcome.

MRI to Detect Hemophilic Arthropathy

Arthropathy can be prevented by routine replacement of FVIII in patients with hemophilia A. However, whether prophylaxis retains efficacy if it is initiated after the onset of recurrent hemarthroses or in individuals with established arthropathy (secondary or tertiary prophylaxis) is unknown.

At the ASH meeting, Gliorioso et al presented the results of a meta-analysis that examined the natural history of hemophilic arthropathy in patients with hemophilia A who were treated only in response to acute bleeding (on-demand therapy) as a baseline for future work. The researchers sought to estimate the amelioration of arthropathy attributable to secondary or tertiary prophylaxis.

Data were aggregated from participants given on-demand therapy during three studies—the JOS19 and JOSc20, baseline results from the SPINART study,21 and the Cross-Sectional MRI Study22—into a single database. To be eligible, patients had to have a FVIII level ≤ 2%; a negative history for inhibitors; a bleeding history of ≥ 12 months before study data acquisition; and assessments of both ankles, knees, and elbows. Joints were assessed using physical examination and T2 gradient echo MRI scored using the World Federation of Hemophilia Gilbert score and the 45-point extended MRI Scales. MRI data were divided into soft-tissue and osteochondral changes. Bleeding episodes and Gilbert scores for all six joints and MRI data for knees and ankles were analyzed using standard descriptive statistics; regression methods were used to quantify the rate of change in scores of joint damage with age.

Results from the three studies provided data on 275 patients between 1 and 50 years of age. Clinical findings, Gilbert scores, and MRI data were extracted and analyzed from multiple joints in each of 157 individuals given on-demand treatment only. The number of bleeding episodes was approximately constant at 20 per year, regardless of age. Gilbert scores showed a steeper rise in young patients but did not increase as patients passed the late-teenage years. However, when compared with bleeding rates and Gilbert scores, MRI scores showed continued age-related deterioration. Age-related total and osteochondral deterioration on MRI increased steadily with age (on average, 2 MRI points per year of age; P > 0.0001). Soft-tissue scores increased rapidly to approximately 9 by age 20 years and then plateaued at approximately 10 for ages 20–50 years (0.08 MRI points per year; P = 0.28).

The authors of this study concluded that individual patient meta-analysis of bleeding frequency, joint physical examination, and MRI joint structure are useful for determining the natural history of hemophilic arthropathy in patients receiving on-demand therapy. Joint bone and cartilage abnormalities increased continuously across the age span despite an early leveling in bleeding rate and joint physical function. These data will be critical for determining the quantitative effects of prophylaxis begun at various ages on mitigation of joint deterioration following the onset of joint bleeding.

Reducing the Incidence of Inhibitors

Early Low-dose Prophylaxis in the Absence of Immunologic Danger Signals

The development of inhibitors is regulated by patient-specific and treatment-related factors associated with the immune response. Of the known risk factors, intensive treatment at an early age has been shown to be significant. Early prophylaxis, defined as first exposure to FVIII in the absence of a bleed in the first year of age, may induce FVIII tolerance, thereby protecting patients from inhibitor development.

The Early Low-Dose Prophylaxis in the Absence of Immunologic Danger Signs (EPIC) Study prospectively assessed the ability of a once-weekly, low-dose prophylactic regimen started before 1 year of age and before the onset of a severe bleeding phenotype (ie, joint bleed) combined with the minimization of immunologic danger signals to reduce the incidence of inhibitor formation in previously untreated patients with severe and moderately severe hemophilia A.

This study enrolled 22 patients, 19 of whom received treatment with FVIII. All patients had severe hemophilia A. Eleven (58%) of these patients were never exposed to factor VIII before, whereas the remaining 42% previously were treated with FVIII concentrates. Eight subjects developed a confirmed inhibitor, and two of these individuals had only borderline positivity at inhibitor testing. Thus, the incidence of an inhibitor titer > 0.6 Bethesda units (BU) in previously untreated patients was 27%. However, 67 major protocol deviations were reported in 15 patients; 44 deviations were reported in 10 patients and were related to the treatment...
regimen. These deviations contrasted with the protocol intention, which was
to minimize immunologic danger signals and low-dose prophylactic regimens. As a
result of the observed inhibitor incidence, the study was terminated based on a futility
analysis (the probability to achieve the primary endpoint of inhibitor rate reduction
to ≤15%).

A significant number of violations in the setting of a demanding study protocol
made it difficult to determine whether treatment decisions in the presence of
immunologic danger signals correlate with inhibitor formation. Thus, the investi-
gators could not verify or disprove the hypothesis that early low-dose prophyla-
xis given in the absence of immunologic danger signals might reduce inhibitor
incidence in previously untreated patients with hemophilia A.

Prompt Immune Tolerance Induction at Inhibitor Diagnosis

Immune tolerance induction (ITI) for patients with hemophilia A with inhibitors has an overall reported success rate of 60%-80%, yet it is the only modality
currently known to effectively eradicate inhibitors. One debate concerns the optimal time to
start ITI. Recent guidelines recommend delaying ITI until the inhibitor titer is
<10 BU.23

Nakar et al24 performed an analysis to determine the success of ITI relative to
time from inhibitor detection to ITI initiation. Data were collected retrospectively
from two hemophilia centers in the United States. Patients with severe or
moderate (≤5%) FVIII deficiency underwent ITI; investigators gathered information on the time interval from inhibitor detection to ITI start, inhibitor titer, and outcome. High-dose ITI was practiced
by both centers at ≥100 IU/kg per day. Success, partial success, and failure were defined pragmatically, with success involving a negative inhibitor titer and the ability to
use FVIII concentrate routinely to treat and prevent bleeding. Partial success
involved an inhibitor titer < 5 BU with the ability to use FVIII concentrate to treat
bleeding episodes. Failure was defined as ongoing ITI > 3 years without achieving
success or partial success or as discontinuation of ITI.

Patients were first divided into a low-
responding inhibitor (LRI) subgroup and a high-responding inhibitor (HRI)
subgroup based on peak inhibitor titer. The HRI subgroup was further subdivided
based on the time to ITI initiation; the first subgroup initiated therapy within 1
month, the second from 1 to 6 months, and the third >6 months from diagnosis
of the inhibitor. The HRI subgroup starting ITI within 1 month was analyzed based on pre-ITI inhibitor titers.

This analysis included male patients
with adequate ITI history documentation. Almost all (95%) had severe FVIII
deficiency. Overall, 49 of 58 patients (84%) underwent successful ITI. Among those
with LRIs (19 patients [33%]), ITI success was 100%. Among 39 patients (67%)
harboring HRIs, tolerance was achieved in 30
(77%), and partial success and continued
ITI was achieved in 1 individual (3%); therapy failed in 7 (18%).

Of 23 patients who started ITI within 1
month of detection, 91% achieved success. All 13 patients (100%) starting ITI with a
pre-ITI inhibitor titer ≥10 BU achieved success. Of the 11 patients who started
ITI 6 months after detection of inhibitor, only 7 (64%) achieved success; therapy
failed in 4 (36%).

These results suggest that the time in-
terval from inhibitor detection to the start
of ITI may play a critical role in eventual
outcome. An inhibitor titer ≥10 BU did not influence outcome when ITI was used
within 1 month of detection, supporting this approach in contrast to the commonly
accepted practice of delaying the start
of ITI until an inhibitor titer <10 BU is achieved. Patients with hemophilia A
may benefit from prompt ITI regardless of the current inhibitor titer and are not
subjected to wait periods when bleeding is more likely to occur. Prompt ITI should be
considered a worthwhile therapeutic op-
tion in newly identified inhibitor patients irrespective of inhibitor titer.

MANAGEMENT OF ACUTE BLEEDING

Four-Factor Prothrombin Complex in Achieving Hemostasis

When rapid correction of coagulopa-
thy is required, as in cases of intracranial
hemorrhage, administration of prothrombin complex concentrate (PCC) may offer
some advantages over infusion of fresh
frozen plasma. Historically, the major
drawback of PCCs has been the risk of
thrombotic complications.25 However,
PCCs have many benefits. For example,
they are subjected to at least one viral in-
activation step, which reduces the risk of
pathogen transmission. Further, they are
nanofiltered, so they are unlikely to pro-
voke transfusion-related acute lung injury.

A four-factor prothrombin complex
concentrate (4F-PCC) recently was
approved by the US Food and Drug
Administration for urgent vitamin K
antagonist (VKA) reversal in patients
with major bleeding. Rapid reversal of
VKA-induced anticoagulation is often
needed for patients requiring urgent
surgical procedures. Plasma currently is
the standard of care in the United States
for VKA reversal prior to emergency
surgery.

A prospective phase 2b, open-label,
noninferiority trial compared the effi-
cacy and safety of 4F-PCC with those of
plasma in patients requiring VKA reversal
before undergoing an urgent surgical
procedure.26 Patients were randomized
to receive a single dose of either 4F-PCC
or plasma. Dose was based on baseline
international normalized ratio (INR)
and weight. There were two primary
defaults: effective hemostasis (preven-
tion of excessive perioperative bleeding
from the start of study product infusion.
until the end of the procedure) and rapid INR reduction (≤ 1.3 at 30 minutes after the infusion ended). Effective hemostasis was defined as the actual blood loss not exceeding the predicted blood loss by 30% and 50 mL, “normal” or “mildly abnormal” hemostasis (subjectively assessed), and no administration of non-study coagulation products.

Effective hemostasis was achieved in 89.7% of patients in the 4F-PCC group versus 75.3% of the plasma group, demonstrating both noninferiority and superiority of 4F-PCC over plasma (14.3% difference; 95% confidence interval = 2.8, 25.8). Likewise, rapid INR reduction was achieved in 55.2% of the 4F-PCC group versus 9.9% of the plasma group, demonstrating noninferiority and superiority of 4F-PCC over plasma. Mortality, the frequency of serious adverse events, and that of thromboembolic events were similar between the groups. Also, significantly fewer fluid overload events occurred with 4F-PCC than with plasma.

Further phase 3 studies evaluating the safety and efficacy of 4F-PCC should be pursued in patients with hemophilia with and without inhibitors.

**CONCLUSION**

Over the past 50 years, the lifespan of an individual affected with severe hemophilia has increased from a mere 20 years to near that of the general unaffected population. This improvement is a result of parallel advances in the development and manufacturing of safe and effective coagulation factor replacement therapies and prophylactic regimens. Furthermore, these strides are products of technologic leaps that allow for rapid control of acute, life-threatening bleeds. Ongoing studies will be crucial in continually improving the quality of life of people with hemophilia and decreasing the cost of their care.

**REFERENCES**

CME/CNE Post Test

Using these two pages as a worksheet, select the best answer to each question based on your reading of the articles in this issue of *The Hemophilia Report*, then complete the evaluation on page 44 and see the instructions below it to obtain CME or CNE credit.

1. The most common morbidity seen among patients with severe hemophilia A or B is:
   a. Splenomegaly
   b. Spontaneous soft-tissue bleeding
   c. Hemophilic arthropathy
   d. Hemarthroses

2. The most significant risk factor predisposing patients with hemophilia A to developing alloimmune inhibitory antibodies to coagulation replacement factor therapy is:
   a. Type of F8 gene mutation
   b. Age
   c. Caucasian race
   d. Prolonged use of prophylactic therapy

3. Which of the following statements comparing patients with hemophilia B to those with hemophilia A is true?
   a. Patients with hemophilia B have a higher risk of recurrent hemarthrosis and, consequently, undergo more frequent total joint arthroplasty than patients with hemophilia A.
   b. Patients with hemophilia B receiving prophylactic factor IX therapy require more frequent doses than patients with hemophilia A receiving prophylactic factor VIII therapy.
   c. Patients with hemophilia B are more likely to develop inhibitory antibodies to prophylactic replacement factor therapy than patients with hemophilia A receiving prophylaxis.
   d. Patients with hemophilia B have less severe gene mutations than patients with hemophilia A and more missense mutations than null mutations.

4. Analysis of data from patients undergoing counseling prior to genetic testing revealed that approximately _______ of cases of hemophilia are sporadic.
   a. 15%
   b. 30%
   c. 45%
   d. 60%

5. Vietri and colleagues reported that in a study of adults and children with moderate-to-severe hemophilia A or B, poorer adherence to therapy was significantly associated with all of the following except:
   a. Physical health status in adults
   b. Physical health status in children
   c. An increase in bleeding episodes requiring replacement factor therapy in adults
   d. Number of work or school days missed because of bleeding episodes in adults and children

6. Development of inhibitors renders factor replacement ineffective in up to 30% of patients with hemophilia A and _______ of patients with hemophilia B.
   a. 1%–2%
   b. 3%–5%
   c. 10%–15%
   d. 20%–30%

7. Investigators at University College London documented post–gene transfer factor IX (FIX) levels of 5%–10% and stable FIX levels beyond 30 weeks with adeno-associated virus-mediated delivery of an F9 transgene, although patients experienced:
   a. Ketoadenosis
   b. Severe pyrexia
   c. Mild transaminitis
   d. Adenovirus infections

8. Patients with hemophilia A receiving prophylactic therapy with recombinant factor VIII (FVIII) who have prolonged intervals of circulating FVIII levels below _______ risk an increase in bleeding episodes and hemarthroses.
   a. 1 IU/dL
   b. 2 IU/dL
   c. 3 IU/dL
   d. 4 IU/dL
9. Results of the Joint Outcome Study in children with severe hemophilia A:
   a. Proved the superiority of episodic FVIII therapy over prophylactic FVIII therapy
   b. Established the superiority of prophylactic FVIII therapy over episodic FVIII therapy
   c. Were inconclusive as to whether episodic FVIII therapy or prophylactic FVIII therapy was superior
   d. Were limited to physical examination of both ankles, knees, and elbows

10. The only treatment modality known to effectively eradicate immunologic inhibitors in patients with hemophilia A is:
    a. Infusion of four-factor prothrombin complex concentrate
    b. Early low-dose prophylaxis given in the absence of immunologic danger signals
    c. Immunosuppression with systemic corticosteroids and cyclophosphamide
    d. Immune tolerance induction
Evaluation

Your candid and thorough completion of this evaluation will help us improve the quality of our CME/CNE activities. Thank you for your participation.

1. As a result of this activity, I am more knowledgeable about the …
   a. Genetic basis of hemophilia A and B and the differences between the two types of hemophilia.
   b. History of hemophilia management, current best practices in children and adults, and the goals of ongoing research to better understand the disease.
   c. Developments being made in the laboratory and currently being tested in clinical trials to produce synthetic coagulation factors with longer half-lives than those of present blood products for treating hemophilia or that are less prone to stimulate the production of neutralizing antibodies (inhibitors).
   d. Advantages and drawbacks of prophylactic versus on-demand therapy, the controversies over therapeutic timing and individualizing therapy for different patients, the factors affecting patient compliance, and current strategies to individualize patient management.

2. I found the content of this educational activity …
   a. Clearly written and well organized.
   b. Accurate and timely.
   c. Related to its overall objectives.
   d. Free from commercial bias.
   e. Relevant to my own clinical practice.

3. Did the information you received from this CME/CNE activity:
   a. Confirm the way you currently manage your patients?
   b. Suggest new options for managing your patients that you might apply in the future?

4. I used the information in this CME/CNE activity for … (check all that apply)
   a. Patient management
   b. Board review
   c. CME/CNE credit

5. Approximately how long (in hours) did it take you to complete this activity, including this evaluation?
   _______ hours

Instructions for Obtaining CME or CNE Credit

To receive CME or CNE credit for completing this free educational activity and a certificate from the CME/CNE provider:

- Study the educational material presented in this issue of The Hemophilia Report.
- Using pages 42–43 as a worksheet, answer all of the post-test questions based on the content of this issue.
- Visit www.HemophiliaReport.com on the Web by May 1, 2015 (for CME credit) or May 1, 2016 (for CNE credit), click CME/CNE Credit, read the information provided, and then click the appropriate link for physicians or nurses to apply for credit and take the post test and evaluation.
- The full text of each article is available at the HemophiliaReport.com Web site, should you need to refer to it again.