emophilia 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.1 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.1–3

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.4–6

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.7 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 $150,000.5 Because of this, it is estimated that two thirds of the world do not have proper access to replacement therapies.5,5

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TABLE 1
Past, Present, and Future of Hemophilia Treatment

<table>
<thead>
<tr>
<th>Decade</th>
<th>Year</th>
<th>Main events</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1977</td>
<td>Desmopressin</td>
</tr>
<tr>
<td>1980s: many shadows, a few lights</td>
<td>1982</td>
<td>Factor IX gene (F9) cloned&lt;br&gt;Acquired immunodeficiency syndrome (AIDS) recognized</td>
</tr>
<tr>
<td></td>
<td>1983</td>
<td>Early virucidal methods (dry-heating)</td>
</tr>
<tr>
<td></td>
<td>1984</td>
<td>Factor VIII gene (F8) cloned&lt;br&gt;Human immunodeficiency virus (HIV) isolated</td>
</tr>
<tr>
<td></td>
<td>1985</td>
<td>Anti-HIV testing</td>
</tr>
<tr>
<td></td>
<td>1987</td>
<td>Safe virus-inactivated plasma factors</td>
</tr>
<tr>
<td></td>
<td>1989</td>
<td>Recombinant factor VIII</td>
</tr>
<tr>
<td>1990s: a new golden era</td>
<td>1994</td>
<td>Immune tolerance</td>
</tr>
<tr>
<td></td>
<td>1996</td>
<td>Highly active antiretroviral therapy (HAART) for HIV infection&lt;br&gt;Recombinant factor VIIa</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>Recombinant factor IX</td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>Eradication of hepatitis C virus (HCV) infection by interferon-ribavirin</td>
</tr>
<tr>
<td>The next 10 years</td>
<td>2011–2021</td>
<td>More factor concentrate available worldwide&lt;br&gt;Longer-acting recombinant coagulation factors&lt;br&gt;Fusion coagulation factors&lt;br&gt;Cure of hemophilia via gene transfer</td>
</tr>
</tbody>
</table>

Source: Franchini and Mannucci

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).4 When these N-acetyllneuraminic acid polymers are attached to recombinant factor proteins, they attract water and produce a watery “cloud” that surrounds

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**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>
</tr>
</thead>
<tbody>
<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>
</tr>
<tr>
<td></td>
<td>New recombinant</td>
<td>Kogenate PF</td>
<td>Bayer</td>
<td>Phase 3 trial completed; post-marketing surveillance ongoing</td>
</tr>
<tr>
<td></td>
<td>New recombinant</td>
<td>GreenGene F</td>
<td>Green Cross</td>
<td>Phase 3 trial completed</td>
</tr>
<tr>
<td></td>
<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>
</tr>
<tr>
<td></td>
<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>
</tr>
<tr>
<td></td>
<td>Site-specific PEG rFVIII</td>
<td>NB-GP</td>
<td>Novo Nordisk</td>
<td>Phase 1 trial completed; phase 3 trial ongoing</td>
</tr>
<tr>
<td></td>
<td>Random PEG rFVIII</td>
<td>BAX 855</td>
<td>Baxter</td>
<td>Phase 1 trial completed; phase 2/3 trial ongoing</td>
</tr>
<tr>
<td></td>
<td>Fc Fusion rFVIII</td>
<td>rFVIII-Fc</td>
<td>Biogen Idec</td>
<td>Phase 1/2/3 trials completed; pediatric phase 3 trial ongoing</td>
</tr>
<tr>
<td></td>
<td>Single-chain rFVIII</td>
<td>CSL627</td>
<td>CSL Behring</td>
<td>Phase 2/3 trials ongoing</td>
</tr>
<tr>
<td>FIX</td>
<td>New recombinant</td>
<td>IB1001</td>
<td>Cangene</td>
<td>Phase 2/3 trials ongoing</td>
</tr>
<tr>
<td></td>
<td>New recombinant</td>
<td>BAX 326</td>
<td>Baxter</td>
<td>Phase 3 trial completed</td>
</tr>
<tr>
<td></td>
<td>Site-specific PEG rFIX</td>
<td>N9-GP</td>
<td>Novo Nordisk</td>
<td>Phase 1 trial completed; phase 3 trial ongoing</td>
</tr>
<tr>
<td></td>
<td>Fc fusion rFIX</td>
<td>rFIX-Fc</td>
<td>Biogen Idec</td>
<td>Phase 1/2/3 trials completed; pediatric phase 3 trial ongoing</td>
</tr>
<tr>
<td></td>
<td>Albumin fusion rFIX</td>
<td>rFIX-FP</td>
<td>CSL Behring</td>
<td>Phase 1/2 trials completed; phase 2/3 trials ongoing</td>
</tr>
<tr>
<td>FVIIa</td>
<td>New recombinant</td>
<td>BAX 817</td>
<td>Baxter</td>
<td>Phase 3 trial ongoing</td>
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<tr>
<td></td>
<td>New recombinant</td>
<td>BAY 86-6150</td>
<td>Bayer</td>
<td>Phase 1/2 trials completed; phase 2/3 trials ongoing</td>
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<tr>
<td></td>
<td>New recombinant</td>
<td>rhFVIIa</td>
<td>rEVO Biologics</td>
<td>Phase 1 trial completed</td>
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<td></td>
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<td>CSL689</td>
<td>CSL Behring</td>
<td>First-in-human trial completed</td>
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<td></td>
<td>Fusion rFVIIa</td>
<td>rFVIIa-CTP</td>
<td>Prolor Biotech</td>
<td>Upcoming human trials</td>
</tr>
<tr>
<td>Other</td>
<td>Bispecific antibody</td>
<td>hBS23</td>
<td>Chugai</td>
<td>Phase 1 trial ongoing in Japan</td>
</tr>
</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 website10

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**FIGURE 2** Schematic representation of PEGylated liposomes. PEG = polyethylene glycol. Reproduced, with permission, from Peyvandi et al.4
In a first-in-human, dose-escalation study of the fusion protein linking rFVIII 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. 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. Phase 3 trials in adults and children are ongoing.

**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. 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 prophyllactic 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 patients achieved bleeding control with one or two doses. 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. 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.

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. 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). 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.

**Enhanced Interactions with vWF**

The primary determinant of the half-life of FVIII is interaction with vWF, which

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**Figure 3** Schematic representation of polyssialylation. Reproduced, with permission, from Peyvandi et al.

**Figure 4** Schematic representation of crystallizable fragment (Fc) fusion. Reproduced, with permission, from Peyvandi et al.

**Figure 5** Schematic representation of albumin fusion. Reproduced, with permission, from Peyvandi et al.
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 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%.28

**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.29 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 monogenic 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 transaminitis, 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 (ASKBIO0009), 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.35,26 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
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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