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.

**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.

Claar 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. 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 others 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 crystalizable fragment (Fc) fusion protein technology with rFVIII results in an rFVIIIFc fusion protein with an extended half-life (mean, 19 hours). Mahlangu et al 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

**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
requires 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 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 TKA 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 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 others11 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

Ziha et al12 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.

REFERENCES


