CLINICAL TRIALS AND OBSERVATIONS

Phase 3 study of recombinant factor VIII Fc fusion protein in severe hemophilia A

Johnny Mahlangu,[1] Jerry S. Powell,[2] Margaret V. Ragni,[3] Pratima Chowdary,[4] Neil C. Josephson,[5] Ingrid Pabinger,[6] Hideji Hanabusa,[7] Naresh Gupta,[8] Roshni Kulkarni,[9] Patrick Fogarty,[10] David Perry,[11] Amy Shapiro,[12] K. John Pasi,[13] Shashikant Apte,[14] Ivan Nestorov,[15] Haiyan Jiang,[15] Shuanglian Li,[15] Srividya Neelakantan,[15] Lynda M. Cristiano,[15] Jaya Goyal,[15] Jurg M. Sommer,[15] Jennifer A. Dumont,[15] Nigel Dodd,[15] Karen Nugent,[15] Gloria Vigliani,[15] Alvin Luk,[15] Aoife Brennan,[15] and Glenn F. Pierce,[15] for the A-LONG Investigators

[1]Haemophilia Comprehensive Care Centre, Faculty of Health Sciences, University of the Witwatersrand and National Health Laboratory Service, Johannesburg, South Africa; [2]University of California at Davis, Davis, California; [3]University of Pittsburgh and the Hemophilia Center of Western Pennsylvania, Pittsburgh, PA; [4]Katharine Dormandy Haemophilia Centre and Thrombosis Unit, Royal Free Hospital, London, United Kingdom; [5]Puget Sound Blood Center, Seattle, WA; [6]Universitätsklinik für Innere Medizin I, Medizinische Universität Wien, Wien, Austria; [7]Department of Hematology, Ogikubo Hospital, Suginami-ku, Tokyo, Japan; [8]Haemophilia Centre and Department of Medicine, Maulana Azad Medical College & Associated Lok Nayak Jai Prakash Narayan Hospital, New Delhi, India; [9]Department of Pediatrics and Human Development, Michigan State University, East Lansing, MI; [10]Penn Comprehensive Hemostasis and Thrombosis Program, Hospital of the University of Pennsylvania, Philadelphia, PA; [11]Cambridge Haemophilia & Thrombosis Centre, Addenbrooke's Hospital, Cambridge, United Kingdom; [12]Indiana Hemophilia and Thrombosis Center, Department of Hematology, Indianapolis, IN; [13]Royal London Haemophilia Centre, Barts and the London School of Medicine and Dentistry, London, United Kingdom; [14]Sahyadri Hospital, Department of Hematology, Mahara, India; and [15]Biogen Idec Hemophilia, Cambridge, MA

Key Points

- A novel recombinant factor VIII with prolonged half-life, rFVIIIFc, was developed to reduce prophylactic injection frequency.
- rFVIIIFc was well-tolerated in patients with severe hemophilia A, and resulted in low bleeding rates when dosed 1 to 2 times per week.

This phase 3 pivotal study evaluated the safety, efficacy, and pharmacokinetics of a recombinant FVIII Fc fusion protein (rFVIIIFc) for prophylaxis, treatment of acute bleeding, and perioperative hemostatic control in 165 previously treated males aged ≥12 years with severe hemophilia A. The study had 3 treatment arms: arm 1, individualized prophylaxis (25-65 IU/kg every 3-5 days, n = 118); arm 2, weekly prophylaxis (65 IU/kg, n = 24); and arm 3, episodic treatment (10-50 IU/kg, n = 23). A subgroup compared recombinant FVIII (rFVIII) and rFVIIIFc pharmacokinetics. End points included annualized bleeding rate (ABR), inhibitor development, and adverse events. The terminal half-life of rFVIIIFc (19.0 hours) was extended 1.5-fold vs rFVIII (12.4 hours; *P* < .001). Median ABRs observed in arms 1, 2, and 3 were 1.6, 3.6, and 33.6, respectively. In arm 1, the median weekly dose was 77.9 IU/kg; approximately 30% of subjects achieved a 5-day dosing interval (last 3 months on study). Across arms, 87.3% of bleeding episodes resolved with 1 injection. Adverse events were consistent with those expected in this population; no subjects developed inhibitors. rFVIIIFc was well-tolerated, had a prolonged half-life compared with rFVIII, and resulted in low ABRs when dosed prophylactically 1 to 2 times per week. This trial was registered at www.clinicaltrials.gov as #NCT01181128. (*Blood*. 2014;123(3):317-325)

Introduction

Patients with hemophilia A experience frequent life-threatening spontaneous and traumatic bleeding, particularly in joints and muscle; recurrent hemarthrosis is a major cause of morbidity in patients with hemophilia.[1,2] Regular replacement of coagulation factor VIII (FVIII) to prevent bleeding is the standard of care; however, existing FVIII products have short half-lives (~12 hours) necessitating frequent intravenous injections (3-4 times weekly) to maintain protective FVIII levels >1 IU/dL.[3] Extending FVIII half-life can reduce injection frequency, which may lessen treatment burden, improve compliance with prophylaxis, and positively impact therapeutic outcomes.[1,2,4,5]

A protein composed of a single molecule of recombinant FVIII (rFVIII) covalently fused to the Fc domain of IgG_1, recombinant FVIII Fc fusion protein (rFVIIIFc) (efraloctocog alfa), has been developed.[6] Fc fusion prolongs the half-life of rFVIIIFc by utilizing the neonatal Fc receptor and endogenous IgG recycling pathway, which delays lysosomal degradation of IgG and Fc fusion proteins, and cycles them back into the circulation.[2,7] The pharmacokinetics and safety of rFVIIIFc were previously evaluated in a single-dose phase 1/2a study in severe hemophilia A.[8] Here, we report the results of a phase 3 open-label, multicenter, partially randomized study that evaluated the comparative pharmacokinetics of rFVIIIFc and rFVIII, and the safety, tolerability, and efficacy of repeated rFVIIIFc dosing for prophylaxis, treatment of acute bleeding, and perioperative management in previously treated adolescents and adults with hemophilia A.

Submitted September 30, 2013; accepted October 12, 2013. Prepublished online as *Blood* First Edition paper, November 13, 2013; DOI 10.1182/blood-2013-10-529974.

The online version of this article contains a data supplement.

There is an Inside *Blood* commentary on this article in this issue.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

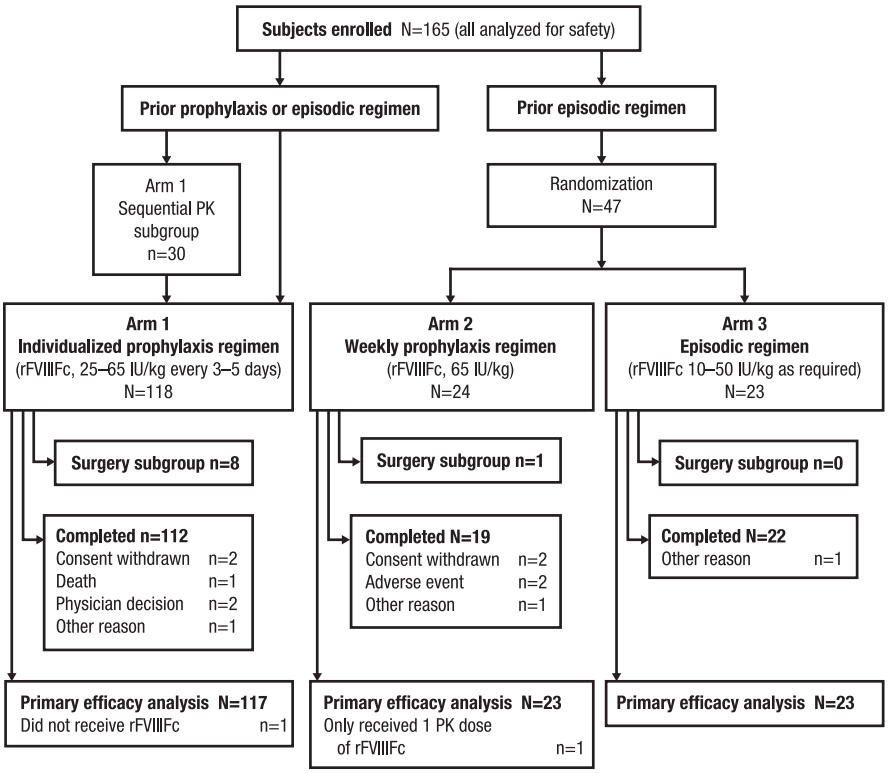

Figure 1. Subject disposition. After screening, all subjects on a prophylactic regimen prior to study entry were to enter into arm 1; subjects on an episodic regimen prior to study entry were to enter into arm 1 or be randomized into either arm 2 or 3, with randomization stratified based on individual annualized bleeding episodes in the prior 12 months. Of the 30 subjects enrolled in the arm 1 sequential pharmacokinetics (PK) group, 28 had evaluable PK profiles for both rFVIII and rFVIIIFc; repeated PK analysis for rFVIIIFc was performed at weeks 12 to 24. Of the 164 subjects in the 3 arms combined, 4 subjects (2.4%) experienced AEs that led to discontinuation of rFVIIIFc treatment and/or withdrawal from the study: rash in 1 subject (assessed as related to rFVIIIFc treatment), femur fracture in 1 subject (assessed as unrelated to rFVIIIFc treatment), death in 1 subject (fatal outcome of polysubstance overdose and completed suicide, assessed as unrelated to rFVIIIFc treatment), and arthralgia in 1 subject (assessed as related to rFVIIIFc treatment, but subject was recorded to have discontinued the study due to withdrawal of consent). Of the 3 subjects who discontinued for "other" reasons, 1 subject was incarcerated, 1 subject was traveling and could not ensure proper temperature conditions for study treatment, and 1 subject was not willing to reveal the reason for wanting to complete the early termination visit.

Methods

Study participants

Previously treated male patients aged ≥12 years with severe hemophilia A (<1 IU/dL [1%] endogenous FVIII activity or severe genotype) were eligible if treated prophylactically, or episodically with a history of ≥12 bleeding events in the 12 months prior to the study. Subjects were excluded if they had a history of inhibitors (neutralizing antibodies), history of hypersensitivity associated with any FVIII concentrate or intravenous immunoglobulin, or other coagulation disorders.

Study design

This was a phase 3 open-label, multicenter, partially randomized study of rFVIIIFc in patients with severe hemophilia A. The protocol was approved by the local institutional review boards/ethics committees, and the study was conducted in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice and the ethical principles outlined in the Declaration of Helsinki. All patients, or patient guardians, gave written informed consent.

The study enrolled 165 subjects into 1 of 3 treatment arms (Figure 1): arm 1, individualized prophylaxis (twice-weekly dosing; 25 IU/kg on day 1 and 50 IU/kg on day 4 to start, followed by 25-65 IU/kg every 3-5 days, n = 118); arm 2, weekly prophylaxis (65 IU/kg, n = 24); or arm 3, episodic (on-demand) treatment as needed for bleeding episodes (10-50 IU/kg, depending on bleeding severity, n = 23). In arm 1, blood samples were collected before the second dose to evaluate pharmacokinetics, including trough levels. Each subject's pharmacokinetic parameters were used to guide individual adjustments to dosing interval (down to 3 days or up to 5 days), and dose (up to 65 IU/kg) to target a steady-state trough FVIII level of 1 to 3 IU/dL or higher as needed to maintain good control of breakthrough bleeding. Doses could also be increased and the interval decreased if the subject experienced 2 spontaneous bleeds in an 8-week period. All subjects on a prophylactic regimen prior to study entry were enrolled into arm 1; subjects on an episodic regimen prior to study entry had the option to enter into arm 1 or be randomized into either arm 2 or arm 3, with randomization stratified based on individual bleeding episodes in the past 12 months. A surgery subgroup included subjects from any arm who required major surgery.

Baseline rFVIIIFc pharmacokinetic measures were evaluated in all subjects. A subgroup of subjects in arm 1 had sequential pharmacokinetic evaluations for comparison with a commercially available rFVIII product (Advate; Baxter, Deerfield, IL). An injection of 50 IU/kg of rFVIIIFc was administered, and pharmacokinetic measures were assessed for 72 hours; following a washout period, an injection of 50 IU/kg of rFVIIIFc was administered, and pharmacokinetic measures were assessed for 120 hours. rFVIIIFc pharmacokinetics were reassessed 12 to 24 weeks later.

Study termination occurred after completion of the specified pharmacokinetic assessments and achievement of the prespecified rFVIIIFc exposure required to ensure acceptable inhibitor detection (eg, a minimum of 104 subjects from any arm with ≥50 exposure days [EDs] to rFVIIIFc; see "Statistical analysis" section). Trough and peak levels of rFVIIIFc were checked for all subjects at each visit to verify subjects maintained targeted troughs.

Outcome measures

The primary efficacy end points were the per-patient annualized bleeding rate (ABR) in arm 1 vs arm 3 and assessment of FVIII activity based on primary pharmacokinetic parameters. Primary safety endpoints were inhibitor development (confirmed by Nijmegen-modified Bethesda assay titer ≥0.6 BU/mL) and adverse events (AEs). Secondary efficacy end points included ABR in arm 2 vs arm 3, and the number of injections and dose per injection of rFVIIIFc required to resolve a bleeding episode. Comparative pharmacokinetics for rFVIIIFc and rFVIII were evaluated using a 1-stage clotting (activated partial thromboplastin time) assay, calibrated to normal human reference plasma with potency traceable to World Health Organization standards; the 2-stage chromogenic activity assay was used for confirmatory pharmacokinetics analysis. Additional immunogenicity assessments were performed in accordance with regulatory guidance,[9,10] including assessment of nonneutralizing antibodies (NNAs) by an electrochemiluminescence-based anti-rFVIIIFc binding-antibody assay. Investigators' assessments of subjects' response to surgery were also evaluated.

Table 1. Subject demographics and baseline characteristics

	Arm 1: individualized prophylaxis (n = 118)	Arm 2: weekly prophylaxis (n = 24)	Arm 3: episodic treatment (n = 23)	Total (n = 165)
Age, y, median (min, max)	29 (12, 65)	31.5 (18, 59)	34 (13, 62)	30 (12, 65)
Weight, kg, median (min, max)	71.65 (42.0, 127.4)	75.85 (50.0, 105.0)	70.00 (48.0, 110.4)	71.60 (42.0, 127.4)
Race, n (%)				
White	79 (66.9)	12 (50.0)	16 (69.6)	107 (64.8)
Black	7 (5.9)	1 (4.2)	2 (8.7)	10 (6.1)
Asian	27 (22.9)	11 (45.8)	5 (21.7)	43 (26.1)
Other	5 (4.2)	0 (0)	0 (0)	5 (3.0)
Geographic location, n (%)				
Europe	34 (28.8)	3 (12.5)	4 (17.4)	41 (24.8)
North America	44 (37.3)	5 (20.8)	7 (30.4)	56 (33.9)
Other*	40 (33.9)	16 (66.7)	12 (52.2)	68 (41.2)
Genotype, n (%)				
Intron 22 inversion	41 (35.0)	7 (33.3)	9 (39.1)	57 (35.4)
Frameshift	24 (20.5)	4 (19.0)	6 (26.1)	34 (21.1)
Missense mutation	22 (18.8)	4 (19.0)	1 (4.3)	27 (16.8)
Nonsense mutation	19 (16.2)	6 (28.6)	1 (4.3)	26 (16.1)
Splice site change	7 (6.0)	0 (0)	4 (17.4)	11 (6.8)
Intron 1 inversion	3 (2.6)	0 (0)	1 (4.3)	4 (2.5)
Duplication	1 (0.9)	0 (0)	0 (0)	1 (0.6)
NA	0 (0)	0 (0)	1 (4.3)	1 (0.6)
VWF antigen, IU/dL, median (IQR)	118.0 (85, 151)	129.0 (86, 166)	131.0 (83, 155)	118.0 (85, 153)
Prestudy FVIII regimen, n (%)				
Prophylaxis	87 (73.7)	0 (0)	0 (0)	87 (52.7)
Episodic	31 (26.3)	24 (100)	23 (100)	78 (47.3)
Estimated bleeding events in prior 12 mo, median (IQR)†				
Prior prophylaxis	6.0 (2, 15)	—	—	6.0 (2, 15)
Prior episodic	27.0 (17, 41)	29.5 (19, 44)	24.0 (15, 36)	27.0 (18, 40)
1 or more target joint, n (%)				
Prior prophylaxis	47 (39.8)	—	—	47 (28.5)
Prior episodic	26 (22.0)	22 (91.7)	18 (78.3)	66 (40.0)
HIV-positive, n (%)	25 (21.2)	4 (16.7)	7 (30.4)	36 (21.8)
HCV-positive, n (%)	55 (46.6)	14 (58.3)	13 (56.5)	82 (49.7)

—, none; HCV, hepatitis C virus; NA, not applicable.
*Other included Australia, New Zealand, Brazil, Hong Kong, India, Japan, Russia, and South Africa.
†Calculation was based on available data.

Statistical analysis

Efficacy analyses were performed on data from all subjects who received ≥1 dose of rFVIIIFc. Safety analyses were conducted on data from subjects who received ≥1 dose of either rFVIII or rFVIIIFc. ABRs were calculated based on the number of bleeding episodes during the efficacy period and duration of time that subjects were evaluated for bleeding. Descriptive statistics included median and interquartile range (IQR) ABR values for each arm. Comparisons between each of arms 1 and 2 (prophylactic regimens) and arm 3 (episodic treatment) were based on ABR estimates from a negative binomial regression model. A negative binomial model was preferred over a Poisson regression model as overdispersion of the data were identified; estimates from a Poisson model are unreliable when overdispersion occurs. rFVIIIFc and rFVIII pharmacokinetic parameters were compared using a repeated-measures analysis of variance model with variables for study treatment and subject; 95% confidence intervals (CIs) were provided for geometric means for each parameter. All tests were performed at the 2-sided 0.05 significance level.

Because of the limited number of patients in the hemophilia A population, study sample size was determined using statistical and clinical considerations in accordance with regulatory guidance for adequate demonstration of acceptable inhibitor risk in clinical trials of previously treated FVIII patients, which allows 2 or fewer of 50 subjects to develop an inhibitor, with each subject requiring a minimum of 50 EDs to the investigational drug.[9] Thus, the study was sufficiently powered to exclude an estimated inhibitor incidence of ≥6.8% using an exact Clopper-Pearson 2-sided CI.[11] At this sample size, the study was also powered to detect with 90% power a clinically meaningful difference in ABR of 60% (arm 1 vs arm 3). Further information on the study design can be found in the supplemental Data (available on the *Blood* Web site).

Role of the funding source

Data were collected by the investigators and analyzed by the sponsor (N.D. of Biogen Idec performed the statistical analyses); all authors had access to the final data, participated in data analysis and interpretation, and vouched for the completeness and accuracy of the data. The first draft of the manuscript was cowritten by A.B., G.F.P., and G.V. of Biogen Idec, with input from all coauthors and assistance from a medical writer funded by the sponsor. All authors participated in revising subsequent drafts of the manuscript and made the final decision to submit it for publication.

Results

Patient population

Overall, 165 subjects were enrolled from 60 centers in 19 countries from November 29, 2010 to July 12, 2012; a total of 153 (92.7%) subjects completed the study. Demographics and baseline characteristics were representative of a severe hemophilia A population and are presented in Table 1. Most subjects had a genotype associated with an increased risk of inhibitor development[12-14] (35.4% had

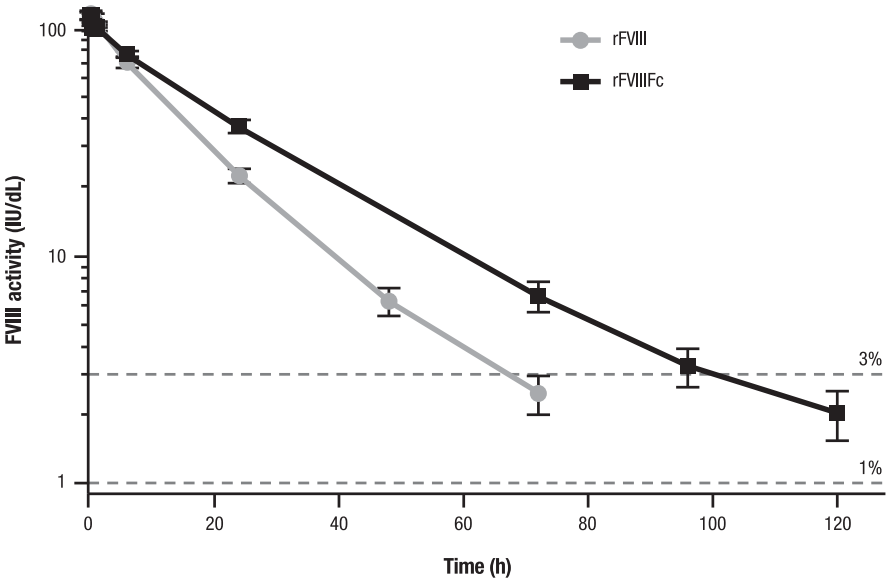

Figure 2. FVIII activity vs time profile for rFVIIIFc and rFVIII, 50 IU/kg intravenous injection. Data presented are observed FVIII activity (mean ± standard error) for each treatment group at nominal times. Dashed lines indicate 1 IU/dL (1%) and 3 IU/dL (3%) trough levels. Pharmacokinetic (PK) parameters were assessed after the first dose of rFVIII or rFVIIIFc for 28 evaluable subjects with data available for both rFVIIIFc and rFVIII. The terminal half-life of rFVIIIFc was significantly longer than that of rFVIII (geometric mean: 19.0 vs 12.4 hours, respectively; $P < .001$). Clearance (95% CI) was significantly lower for rFVIIIFc (2.0 [1.7-2.2] mL/h/kg) vs rFVIII (3.0 [2.7-3.4] mL/h/kg); $P < .001$. Dose-normalized area under the curve (95% CI) was 51.2 (45.0-58.4) and 32.9 (29.3-36.9) IU × h/dL per IU/kg for rFVIIIFc and rFVIII, respectively ($P < .001$). Times to 1 and 3 IU/dL above baseline (95% CI) were 4.9 (4.4-5.5) and 3.7 (3.3-4.1) days for rFVIIIFc vs 3.3 (3.0-3.7) and 2.5 (2.2-2.7) days for rFVIII, respectively ($P < .001$). h, hours.

intron 22 inversions, 16.1% had nonsense mutations). For subjects on prior prophylaxis (n = 87), 87% reported injecting ≥3 times weekly on their prior regimen. Subjects on prior episodic treatment had a higher median number of bleeding episodes in the 12 months prior to the study compared with subjects on prior prophylaxis. Among subjects on prior episodic treatment, the per-subject median number of bleeding episodes in the 12 months prior to study entry was similar in those selecting arm 1 compared with those opting to be randomized to arm 2 or arm 3 (27.0 vs 29.5 and 24.0, respectively). A higher proportion of subjects in arms 2 and 3 had target joints.

The median (minimum, maximum) durations of treatment with rFVIIIFc in arms 1, 2, and 3 were 32.1 (9, 54), 28.0 (<1, 38), and 28.9 (15, 32) weeks, respectively. In total, 111 (67.7%) subjects had ≥50 EDs to rFVIIIFc. A total of 9356 rFVIIIFc injections were administered during the study, corresponding to 9170 EDs (100.2 total years on rFVIIIFc). Overall, 93.6% of subjects in the prophylaxis arms were compliant with both the prescribed dose and interval.

Pharmacokinetic parameters

Comparative pharmacokinetic data (compartmental analysis of the 1-stage clotting assay) for rFVIIIFc vs rFVIII were available for 28 of 30 subjects in the sequential pharmacokinetics subgroup (supplemental Table 1). The terminal half-life of rFVIIIFc was significantly longer than that of rFVIII (geometric mean: 19.0 vs 12.4 hours, respectively; $P < .001$) (Figure 2). rFVIIIFc half-life was positively correlated with baseline von Willebrand factor (VWF) antigen concentration (n = 155; $r = 0.67$; $P < .001$). Incremental recovery was similar for rFVIIIFc and rFVIII (2.2 and 2.4 IU/dL per IU/kg, respectively; $P = .025$). Time to 1 IU/dL (1%) FVIII trough level above baseline was longer for rFVIIIFc than for rFVIII (4.9 vs 3.3 days, respectively; $P < .001$) (Figure 2). Comparative pharmacokinetic data obtained using the 1-stage clotting assay were confirmed by the 2-stage chromogenic assay, and rFVIIIFc pharmacokinetics were consistent following repeated dosing over 14 weeks (data not shown).

Efficacy

The ABR was significantly reduced with prophylaxis by 92% (arm 1) and 76% (arm 2) compared with episodic treatment, based on estimates from a negative binomial regression model (2.91, 8.92, and 37.25 for arms 1, 2, and 3, respectively; $P < .001$) (Table 2). The median (IQR) observed ABRs in arms 1, 2, and 3 were 1.6 (0.0, 4.7), 3.6 (1.9, 8.4), and 33.6 (21.1, 48.7), respectively (Table 2). The estimated ABR calculation in arm 2 was substantially influenced by 4 subjects with high individual on-study bleeding rates. These subjects were previously treated episodically and had reported between 23 and 34 bleeding episodes in the year prior to study entry; however, there was no baseline disease or demographic characteristic directly associated with their higher ABR. In arms 1 and 2, 45.3% (53/117) and 17.4% (4/23) of subjects, respectively, had no bleeding episodes; all subjects receiving episodic treatment (arm 3) experienced bleeding episodes.

Among subjects receiving individualized prophylaxis (arm 1), over the last 3 months on the study, the median dosing interval was 3.50 days (mean, 3.87 days) and the median weekly dose was 77.70 IU/kg. Approximately 30% of subjects who were on the study for ≥6 months achieved a dosing interval of at least 5 days in the last 3 months on the study (Tables 2 and 3). Thirty-one of 118 subjects (26.3%) in arm 1 required no adjustment to their initial twice-weekly dosing regimen (25 IU/kg on day 1, 50 IU/kg on day 4 to start) over the course of the study; 65 subjects (55.1%) required 1 adjustment. The majority of dosing adjustments were made after subjects' initial pharmacokinetic review, with 12 of the adjustments made in response to bleeding episodes.

The reduction in ABR achieved by subjects on prophylactic regimens relative to subjects treated episodically was consistent across all prespecified demographic and disease-based subgroups (Figure 3). In the subgroup of arm 1 and arm 2 subjects who were previously treated episodically, a negative binomial model comparing 12-month historical patient-reported bleeding events vs on-study ABR demonstrated a 96% (arm 1) and 71% (arm 2) relative reduction in bleeding events (supplemental Figure 2), similar to the ABR reductions of 92% and 76% observed overall in arms 1 and 2, respectively, compared with arm 3.There was no significant difference in the number of prestudy vs on-study bleeding episodes for subjects in arm 3 (episodic treatment).

Across all arms, 757 bleeding episodes were treated with rFVIIIFc during the efficacy period. Overall, 87.3% of bleeding episodes were resolved with 1 injection, and 97.8% were controlled with ≤2

Table 2. Efficacy end points for prophylaxis and episodic treatment groups

	Arm 1: individualized prophylaxis (n = 117)	Arm 2: weekly prophylaxis (n = 23)	Arm 3: episodic treatment (n = 23)
ABR, negative binomial model (95% CI)	2.9 (2.3-3.7)	8.9 (5.5-14.5)	37.3 (24.0-57.7)
% Reduction vs arm 3 (*P*)*	92 (<.001)	76 (<.001)	—
ABR by type and location of bleeds, median (IQR)			
Overall	1.6 (0.0, 4.7)	3.6 (1.9, 8.4)	33.6 (21.1, 48.7)
Spontaneous	0.0 (0.0, 2.0)	1.9 (0.0, 4.8)	20.2 (12.2, 36.8)
Traumatic	0.0 (0.0, 1.8)	1.7 (0.0, 3.3)	9.3 (1.7, 11.9)
Joint			
Spontaneous	0.0 (0.0, 1.7)	0.0 (0.0, 3.8)	18.6 (7.6, 29.6)
Traumatic	0.0 (0.0, 1.2)	0.0 (0.0, 2.0)	3.9 (0.0, 8.6)
Muscle			
Spontaneous	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	5.1 (1.8, 6.8)
Traumatic	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 2.0)
Subjects with no bleeding episodes, n (%)	53 (45.3)	4 (17.4)	0 (0)
Weekly dose, IU/kg, median (min, max)			
Overall	77.9 (54.0, 141.5)	65.6 (59.4, 70.7)	—
Weekly dose, IU/kg, mean (SD)			
Overall	85.4 (19.3)	65.8 (2.9)	—
Dosing interval during last 3 mo on study,† n (%)			
3 or more d	111 (99.1)	NA	NA
4 or more d	39 (34.8)	NA	NA
5 d	33 (29.5)	NA	NA

—, none; SD, standard deviation; NA, not applicable.
IQR, 25th and 75th percentiles.
*Reduction in ABR compared with arm 3, calculated using negative binomial model.
†In subjects on study for ≥6 months; n = 112 in arm 1, n = 16 in arm 2.

injections (supplemental Table 2). The median dose per injection to treat a bleeding episode was 27.35 IU/kg. For bleeding episodes that required >1 injection for resolution, the median (IQR) interval between the first and second injection was 30.9 (24.5, 49.0) hours.

There were 9 major surgeries performed in 9 subjects, most of which were orthopedic procedures (knee replacement, n = 5; laparoscopic hernia repair, n = 2; arthroscopy, n = 1; appendectomy, n = 1). The hemostatic response during the perioperative period for these major surgeries was rated by investigators/surgeons as excellent (n = 8) or good (n = 1).

Safety

No inhibitors were detected in any subjects with an evaluable inhibitor test, including 110 subjects with ≥50 EDs, for whom the inhibitor incidence was 0% (95% CI, 0% to 3.3%); the inhibitor incidence overall was also 0% (95% CI, 0% to 2.2%). Five subjects tested positive for NNAs at baseline; in all 5 cases, NNA titers declined over the course of the study, such that in 2 subjects, the antibody was not detected at the final visit. Six additional subjects who tested negative at baseline had a positive NNA test on study; a negative confirmatory result was obtained in 4 of these subjects at the final study visit, whereas detection persisted in 2 subjects. All NNAs were low titer and directed against FVIII, not Fc. In 1 subject in arm 1, NNA presence was temporally associated with a transient effect on peak plasma concentration (C_{max}) and clearance at the 14-week pharmacokinetic assessment, which did not impact clinical treatment outcome; the subject continued on to the extension study.

Of the 164 subjects exposed to rFVIIIFc (1 subject received only rFVIII on study), 108 (65.9%) reported ≥1 AE (excluding the perioperative period) (Table 4). The types of AEs were representative of events occurring in the general hemophilia population; the most common (incidence of ≥5% in the combined arms, excluding the perioperative period) were nasopharyngitis, arthralgia, headache, and upper respiratory infection. AEs judged by the investigator to be related to rFVIIIFc treatment occurred in 10 (6.1%) subjects; of these, arthralgia and malaise were reported in more than 1 subject (2 subjects each). Of 164 subjects in arms 1, 2, and 3, 12 subjects (7.3%) had at least 1 serious AE (SAE) during the study (excluding the perioperative period); no SAE was judged to be related to rFVIIIFc treatment. There were no reports of serious vascular thrombotic events, serious hypersensitivity, or anaphylaxis. There was 1 death secondary to polysubstance overdose (suicide) in a subject with prior history of depression, which was assessed as unrelated to rFVIIIFc by the investigator. Safety data obtained during the perioperative period were reviewed separately, and no unique safety concerns were identified.

Discussion

This phase 3 study confirmed previous data on the prolonged clearance-related pharmacokinetics of rFVIIIFc vs rFVIII,[8] and established that rFVIIIFc was well tolerated and efficacious in the prevention and treatment of bleeding in adolescent and adult subjects with severe hemophilia A. This is the first report of phase 3 data for a longer-lasting modified rFVIII in subjects with hemophilia A.

Subjects receiving individualized prophylaxis (arm 1; dosing intervals 3-5 days) and weekly prophylaxis (arm 2) experienced clinically meaningful reductions in ABR with once- or twice-weekly dosing compared with episodic treatment (arm 3). The median ABR in the individualized prophylaxis arm was consistent with ABRs reported for other prophylactic regimens,[15-17] and 45.3% of subjects experienced no bleeding episodes on study. The majority of subjects

Table 3. Summary of prescribed dose and frequency at end of efficacy period, arm 1 (n = 117)

	Dosing frequency, n (%)*				
rFVIIIFc dose, IU/kg	Twice weekly	Every 3 d	Every 4 d	Every 5 d	Total
25	0 (0)	3 (2.6)	0 (0)	0 (0)	3 (2.6)
30	0 (0)	2 (1.7)	0 (0)	0 (0)	2 (1.7)
35	0 (0)	1 (0.9)	0 (0)	0 (0)	1 (0.9)
25/50†	34 (29.1)	0 (0)	0 (0)	0 (0)	34 (29.1)
40	0 (0)	2 (1.7)	0 (0)	0 (0)	2 (1.7)
45	1 (0.9)	0 (0)	0 (0)	0 (0)	1 (0.9)
50	0 (0)	20 (17.1)	2 (1.7)	34 (29.1)	56 (47.9)
60	0 (0)	1 (0.9)	0 (0)	1 (0.9)	2 (1.7)
65	0 (0)	10 (8.5)	2 (1.7)	4 (3.4)	16 (13.7)
Total	35 (29.9)	39 (33.3)	4 (3.4)	39 (33.3)	117 (100)

*The median dosing interval among subjects on study for at least 6 months was 3.5 (range, 2.9-5.7) days when averaged over all dosing intervals during the respective time intervals for the last 3 months on the study.
†Initial twice-weekly dosing regimen of 25 IU/kg on day 1, followed by 50 IU/kg on day 4.

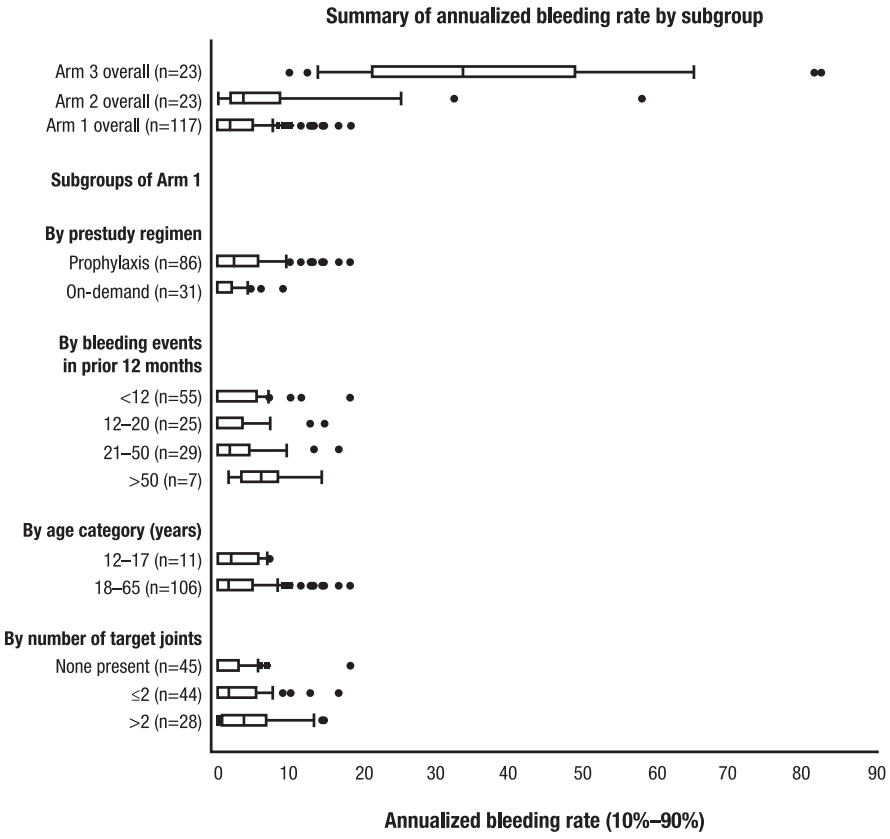

Figure 3. Subgroup analyses of ABRs. Subgroup analyses results were consistent with the primary analyses, showing a reduction in bleeding rates in all prophylaxis subgroups compared with episodic therapy. Arm 1 was the only subgroup with a sufficient number of subjects to display results graphically. The box and whiskers represent the median, 25% to 75% range, and 10% to 90% range, respectively; circles represent individual subject outliers.

(81.4%) in the individualized prophylaxis arm required no adjustment or only 1 adjustment to their dosing regimen over the course of the study, confirming the relative ease with which subjects were transitioned from their previous FVIII regimen to rFVIIIFc.

The neonatal Fc receptor–mediated extension of half-life translated into prolonged clinical activity for rFVIIIFc, reducing dosing frequency to 1 to 2 times weekly (current treatment guidelines recommend dosing 3-4 times weekly[3]). Most subjects on prior prophylaxis reported injecting FVIII ≥3 times weekly prior to entry into arm 1, which would be estimated to require at least 156 injections per year, whereas the on-study median dosing interval in this arm was 3.5 days (ie, twice-weekly dosing; ~104 injections per year), with approximately 30% of subjects who were on study for ≥6 months achieving an interval of 5 days (ie, ~73 injections per year) in the last 3 months on the study. Consistent with the dosing regimens and low ABRs, rFVIIIFc had an increased half-life and longer time above a trough FVIII level of 1 IU/dL (1%) relative to rFVIII for all patients in whom these parameters were evaluated. The prolonged half-life of rFVIIIFc and resultant potential for reduced injection frequency may improve compliance with prophylaxis among patients with hemophilia A, which has the potential to improve both short- and long-term outcomes. The impact of prolonged-interval dosing of rFVIIIFc on compliance requires further investigation.

The interaction between FVIII and VWF in circulation plays a critical role in the half-life of FVIII; VWF protects FVIII from proteolytic degradation and binding to FVIII clearance receptors, and possibly stabilizes FVIII structurally.[18] However, the interaction between VWF and FVIII also limits the ability to extend FVIII half-life, and most strategies studied to date have failed to extend FVIII half-life beyond that of VWF (~10-25 hours[19]).[8,20-23] This finding is supported by the high correlation of VWF levels with rFVIIIFc half-life and is consistent with ~19-hour half-life of rFVIIIFc observed here and in the phase 1/2a study.[8] This same correlation was found, as expected, for rFVIII. Thus, subjects with lower VWF levels exhibited relatively shorter half-lives for both rFVIII and rFVIIIFc, and those with higher VWF levels exhibited relatively longer half-lives for both agents. However, in all cases measured here and in the phase 1/2 study,[8] the comparative pharmacokinetic analyses demonstrated that individual subject half-lives were longer for rFVIIIFc compared with rFVIII.

In contrast to arm 1, the prophylactic regimen for arm 2 was not designed to maintain FVIII activity above 1 IU/dL in all subjects, but rather to provide efficacy data to inform therapeutic decision making for patients unwilling or unable to undertake a more intensive protective regimen. For subjects randomized to arm 2, there was a statistically significant reduction in ABR relative to their prior episodic regimen, and most subjects achieved an ABR in the range reported for existing prophylactic regimens.[15-17] The low median ABR and observation that some subjects (4/23, 17.4%) had no breakthrough bleeding episodes indicate that once-weekly prophylaxis can provide positive clinical outcomes for some patients. However, 3 subjects (13.0%) in this arm experienced >5 bleeding episodes on the study. Thus, although a once-weekly dosing regimen can be considered in select patients, others requiring more aggressive therapy to achieve more comprehensive prophylactic protection from bleeding would be managed on a more intensive dosing regimen.

Overall, rFVIIIFc was well tolerated, as suggested by the high rates of subject retention (92.7%) and overall compliance with the prophylactic regimens (93.6%), and AEs were as expected for the hemophilia A population.[15] There was no evidence of increased immunogenicity with rFVIIIFc, and no subjects developed inhibitors, consistent with the observed lack of increased inhibitor risk when switching between rFVIII products.[24] As inhibitor development is one

Table 4. Summary of AEs*

	Arm 1: individualized prophylaxis (n = 117)	Arm 2: weekly prophylaxis (n = 24)	Arm 3: episodic treatment (n = 23)
Total AEs, n	219	46	23
Subjects with ≥1 AE, n (%)	80 (68.4)	18 (75.0)	10 (43.5)
Most common AEs, ≥3%, n (%)			
Nasopharyngitis	16 (13.7)	1 (4.2)	3 (13.0)
Arthralgia	10 (8.5)	2 (8.3)	1 (4.3)
Upper respiratory tract infection	6 (5.1)	0 (0)	3 (13.0)
Headache	5 (4.3)	6 (25.0)	2 (8.7)
Influenza	5 (4.3)	0 (0)	0 (0)
Pyrexia	3 (2.6)	1 (4.2)	1 (4.3)
Subjects with 1 or more SAE†	10 (8.5)	2 (8.3)	0 (0.0)

*Excludes the perioperative period.

†SAEs in arms 1, 2, and 3 (excluding the perioperative period) included face injury, femur fracture, back pain with associated syncope, hemarthrosis, lumbar spinal stenosis, myalgia, inguinal hernia, tooth disorder, restless leg syndrome, polysubstance overdose and completed suicide, nephrolithiasis, respiratory distress, tachycardia, and hypertensive emergency. The incidence of individual SAEs was 0.6%, with all occurring in 1 subject each. None of the reported 17 SAEs, including 1 death secondary to polysubstance overdose (suicide) in a subject with prior history of depression, were considered by the investigator as related to rFVIIIFc treatment. One SAE (femur fracture) indirectly resulted in discontinuation of rFVIIIFc treatment and withdrawal from the study because the subject required surgery to treat the fracture and did not have the 12 EDs required for enrolling in the surgery subgroup.

of the most serious complications of hemophilia A therapy,[25] clinical studies typically require ≥50 EDs in a predetermined number of subjects for adequate inhibitor assessment.[9] This study included a total of 9170 EDs (100.2 total years on rFVIIIFc), with a substantial proportion of subjects (67.7%) having ≥50 EDs to rFVIIIFc. As this study did not include previously untreated patients, who are at a higher risk of inhibitor development, or subjects with a history of inhibitors to FVIII, further clinical experience will be needed to assess the benefit of rFVIIIFc treatment in these groups.

Largely transient NNAs were observed in a total of 11 (6.7%) subjects (5 at screening; 6 on study), consistent with or lower than previous reports in the inhibitor-negative hemophilia A population[26,27] and similar to the ~5% observed in the normal population. In a recent longitudinal study of the natural history and significance of NNAs in patients with hemophilia A, individual immune responses to FVIII products were variable over time; however, the clinical impact of NNAs in this study remained unclear.[28] In our study, all NNAs were of low titer and had no discernible clinical impact.

Clinical trial design in hemophilia A presents specific challenges, primarily due to the limited availability of patients affected by hemophilia A and ethical considerations that preclude blinded, placebo-controlled studies.[9,29] Given that prophylaxis is the standard of care in many global regions, randomization of a subject from their current prophylactic regimen to an episodic regimen was considered unethical. Thus, this study was partially randomized in that all subjects on a prophylactic regimen prior to study entry were entered into the individualized prophylaxis arm (arm 1), whereas subjects on an episodic regimen prior to study entry could elect to enter arm 1, or be randomized to arm 2 (weekly dosing), or arm 3 (episodic treatment). One consequence of allowing subjects to elect to enter a prophylactic arm is the introduction of self-selection bias. However, the design did not appear to affect the study results; in all prespecified subgroup analyses, including one limited to subjects on a prior episodic regimen, the reduction in ABRs was consistent with the primary analysis across all arms (Figure 3; supplemental Figure 2).

The study was sufficiently powered to compare ABRs in the individualized prophylactic arm to episodic treatment (arm 1 vs arm 3), as well as to assess inhibitor risk; however, it was not designed to compare the individualized and weekly prophylactic regimens (arms 1 and 2, respectively). Thus, although both the individualized (median twice-weekly dosing) and weekly dosing regimens resulted in a significant reduction in ABR compared with episodic treatment, the superiority of one approach for prophylactic dosing over the other cannot be determined from this study. An additional study limitation was the per-protocol maximum 5-day dosing interval in arm 1, which potentially restricted determination of the maximum therapeutic interval for some subjects. Weekly dosing resulted in effective prophylaxis in most subjects in arm 2, suggesting that this could be an appropriate regimen for some patients. Thus, the data presented here support 2 different options for individualized treatment of patients with hemophilia A: a 3 to 5 day dosing interval to maintain trough FVIII levels to support sufficient prophylactic protection, or a longer interval between doses (eg, weekly dosing) to reduce the burden of frequent injections in patients in whom this is appropriate.

This study demonstrated that rFVIIIFc was well tolerated and efficacious in the prevention and treatment of bleeding events, including within the setting of major surgery, in adolescents and adults with severe hemophilia A. The results support the potential for rFVIIIFc dosing 1 to 2 times per week with excellent control of bleeding. Additionally, Fc fusion did not impair FVIII activity or result in increased immunogenicity. Thus, rFVIIIFc offers the potential for an injection frequency reduced by up to 50% compared with existing FVIII therapeutic regimens, an improvement in treatment burden that may increase compliance with prophylaxis among patients with hemophilia A.

Acknowledgments

The authors thank the investigators and all patients who were involved in the A-LONG study; the members of the data and safety monitoring committee, Ekta Seth-Chhabra for the genotype data summary; Feriandas Greblikas for contribution to study oversight; Snejana Krassova for contribution to study oversight, data analysis, and review of manuscript drafts; MaryEllen Carlile Klusacek from Biogen Idec for writing support based on input from authors; James V. Scanlon from Biogen Idec for review of manuscript drafts; Samantha Taylor and Tove Anderson from Evidence Scientific Solutions for their copyediting of the manuscript; and Katie Gersh from MedErgy for styling the manuscript according to the journal requirements; and Biogen Idec for providing review and feedback of the paper to the authors. The authors had full editorial control of the paper and provided their final approval of all content.

This work was supported by funding from Biogen Idec, including funding for the editorial support in the development of this paper.

Authorship

Contribution: G.F.P., A.L., H.J., and K.N. contributed to the design and conceptualization of the research, design of data analyses, interpretation of data, and drafting and revising the manuscript; J.M., S.A., P.C., P.F., N.G., H.H., N.C.J., R.K., I.P., K.J.P., D.P., J.S.P., M.V.R., and A.S. contributed to data collection, design of data

analyses, interpretation of data, and drafting and revising the manuscript; I.N., S.L., L.M.C., S.N., J.G., J.M.S., J.A.D., A.B., and G.V. contributed to the design of data analyses, data collection, interpretation of data, and drafting and revising of the manuscript; and N.D. performed the statistical analyses and contributed to the interpretation of data, and revising the manuscript.

Conflict-of-interest disclosure: J.M. has served as advisor for Amgen, Bayer, and Novo Nordisk and has received funding for clinical trials from Bayer, Novo Nordisk, Biogen Idec, and Inspiration. J.S.P. has received funding for clinical trials from Biogen Idec, Octapharma, Baxter Healthcare, Bayer, CSL Behring, and Novo Nordisk. M.V.R. has served on advisory board for Biogen Idec; has been a consultant for Bristol-Myers Squibb, GlaxoSmithKline, and Tacere Benitec; and has received research support from Baxter, Bayer, Biogen Idec, Bristol-Myers Squibb, CSL Behring, Merck, Novo Nordisk, Pfizer, and GlaxoSmithKline. P.C. has received honoraria and travel grants from Baxter Healthcare, Novo Nordisk, Bayer, Pfizer, CSL Behring, and Biogen Idec; has served on advisory boards for Baxter Healthcare, CSL Behring, and Pfizer; and has received research funding from CSL Behring, Novo Nordisk, and Pfizer. N.J. has conducted clinical research for Biogen Idec, Baxter Healthcare, and Novo Nordisk; has received research support from Bayer and Biogen Idec; and is a paid consultant for Biogen Idec. I.P. has received honoraria from Baxter Healthcare, Bayer, CSL Behring, and Pfizer; and has served on advisory boards for CSL Behring, Novo Nordisk, and Pfizer. H.H. has received honoraria from Baxter Healthcare, Novo Nordisk, Bayer, Pfizer, and KaketsuKen; and has served on advisory boards for Baxter Healthcare. N.G. has received research funding from Biogen Idec. R.K. has served on advisory boards for Biogen Idec and received consulting fees from Biogen Idec. P.F. has received research funding from Baxter Healthcare, Biogen Idec, CSL Behring, and Pfizer; has received honoraria from Bayer Healthcare and NovoNordisk; and has received consulting fees from Baxter Healthcare, Biogen Idec, Grifols, and NovoNordisk. D.P. has received research funding and consulting fees from Biogen Idec. A.S. has served on speakers bureau for Baxter and Novo Nordisk; has served on advisory boards for Baxter, Novo Nordisk, Bayer, and Inspiration; has served on global steering committees for Baxter and Bayer; has received consulting fees from Baxter, Novo Nordisk, and Inspiration; has received research funding from Baxter, Novo Nordisk, Bayer, CSL Behring, Biogen Idec, and Inspiration; and does not personally accept all consulting fees and honorariums, but they are directed to the Indiana Hemophilia and Thrombosis Center. K.J.P. has served on advisory boards for Bayer, BPL, OctaPharma, Biogen Idec, and Pfizer, and has received educational support and travel grants from OctaPharma, Pfizer, Biogen Idec, and Novo Nordisk. I.N., H.J., S.L., S.N., L.M.C., J.G., J.M.S., J.A.D., K.N., A.B., and G.F.P. are employees of and hold equity interest in Biogen Idec. A.L. was employed by and held equity interest in Biogen Idec at the time of this study. G.V. is a paid consultant of and holds equity interest in Biogen Idec. N.D. is a paid consultant of Biogen Idec. The remaining authors declare no competing financial interests.

Correspondence: Glenn F. Pierce, Biogen Idec, 225 Binney St, Cambridge, MA 02142; e-mail: glenn.pierce@biogenidec.com.

References

1. Fogarty PF. Biological rationale for new drugs in the bleeding disorders pipeline. *Hematology Am Soc Hematol Educ Program* 2011;2011:397-404.
2. Rath T, Baker K, Dumont JA, et al. Fc-fusion proteins and FcRn: structural insights for longer-lasting and more effective therapeutics [published online ahead of print October 24, 2013]. *Crit Rev Biotechnol*.
3. National Hemophilia Foundation. MASAC recommendation concerning prophylaxis (regular administration of clotting factor concentrate to prevent bleeding). Available at:http://www.hemophilia.org/NHFWeb/MainPgs/MainNHF.aspx?menuid=57&contentid=1007. Accessed July 18, 2013.
4. Lillicrap D. Improvements in factor concentrates. *Curr Opin Hematol.* 2010;17(5):393-397.
5. Hacker MR, Geraghty S, Manco-Johnson M. Barriers to compliance with prophylaxis therapy in haemophilia. *Haemophilia.* 2001;7(4):392-396.
6. Peters RT, Toby G, Lu Q, et al. Biochemical and functional characterization of a recombinant monomeric factor VIII-Fc fusion protein. *J Thromb Haemost.* 2013;11(1):132-141.
7. Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. *Nat Rev Immunol.* 2007;7(9):715-725.
8. Powell JS, Josephson NC, Quon D, et al. Safety and prolonged activity of recombinant factor VIII Fc fusion protein in hemophilia A patients. *Blood.* 2012;119(13):3031-3037.
9. European Medicines Agency. Guideline on the clinical investigation of recombinant and human plasma-derived factor VIII products. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109692.pdf. Accessed July 18, 2013.
10. United States Food and Drug Administration. Guidance for industry: assay development for immunogenicity testing of therapeutic proteins. Available at: http://www.fda.gov/downloads/Drugs/.../Guidances/UCM192750.pdf. Accessed July 18, 2013.
11. Clopper CJ, Pearson E. The use of confidence or fiducial limits illustrated in the case of binomial. *Biometrika.* 1934;26:404-413.
12. Gouw SC, van den Berg HM, Oldenburg J, et al. F8 gene mutation type and inhibitor development in patients with severe hemophilia A: systematic review and meta-analysis. *Blood.* 2012;119(12):2922-2934.
13. Margaglione M, Castaman G, Morfini M, et al; AICE-Genetics Study Group. The Italian AICE-Genetics hemophilia A database: results and correlation with clinical phenotype. *Haematologica.* 2008;93(5):722-728.
14. Miller CH, Benson J, Ellingsen D, et al; Hemophilia Inhibitor Research Study Investigators. F8 and F9 mutations in US haemophilia patients: correlation with history of inhibitor and race/ethnicity. *Haemophilia.* 2012;18(3):375-382.
15. Valentino LA, Mamonov V, Hellmann A, et al; Prophylaxis Study Group. A randomized comparison of two prophylaxis regimens and a paired comparison of on-demand and prophylaxis treatments in hemophilia A management. *J Thromb Haemost.* 2012;10(3):359-367.
16. Recht M, Nemes L, Matysiak M, et al. Clinical evaluation of moroctocog alfa (AF-CC), a new generation of B-domain deleted recombinant factor VIII (BDDrFVIII) for treatment of haemophilia A: demonstration of safety, efficacy, and pharmacokinetic equivalence to full-length recombinant factor VIII. *Haemophilia.* 2009;15(4):869-880.
17. Manco-Johnson MJ, Kempton CL, Reding MT, et al. Randomized, controlled, parallel-group trial of routine prophylaxis vs. on-demand treatment with sucrose formulated recombinant factor VIII-FS in adults with severe hemophilia A (SPINART). *J Thromb Haemost.* 2013;11(6):1119-1127.
18. Terraube V, O'Donnell JS, Jenkins PV. Factor VIII and von Willebrand factor interaction: biological, clinical and therapeutic importance. *Haemophilia.* 2010;16(1):3-13.
19. Gallinaro L, Cattini MG, Sztukowska M, et al. A shorter von Willebrand factor survival in O blood group subjects explains how ABO determinants influence plasma von Willebrand factor. *Blood.* 2008;111(7):3540-3545.
20. Dumont JA, Liu T, Low SC, et al. Prolonged activity of a recombinant factor VIII-Fc fusion protein in hemophilia A mice and dogs. *Blood.* 2012;119(13):3024-3030.
21. Mei B, Pan C, Jiang H, et al. Rational design of a fully active, long-acting PEGylated factor VIII for hemophilia A treatment. *Blood.* 2010;116(2):270-279.
22. Tiede A, Brand B, Fischer R, et al. Enhancing the pharmacokinetic properties of recombinant factor VIII: first-in-human trial of glycoPEGylated recombinant factor VIII in patients with hemophilia A. *J Thromb Haemost.* 2013;11(4):670-678.
23. Kumar S, Liu T, Kulman JD, et al. Effect of unstructured polypeptide insertions on the recombinant expression of human factor VIII. International Society on Thrombosis and Haemostasis - 24th Congress; 2013; Amsterdam, Netherlands. *J Thromb Haemost.* 2013;11(Suppl. 2):711.

24. Gouw SC, van der Bom JG, Ljung R, et al; PedNet and RODIN Study Group. Factor VIII products and inhibitor development in severe hemophilia A. *N Engl J Med.* 2013;368(3):231-239.

25. Ingerslev J. FDA Workshop on factor VIII inhibitors held at the NIH, Bethesda, MA on 21 November 2003. *Haemophilia.* 2004;10(3):288-289.

26. Whelan SF, Hofbauer CJ, Horling FM, et al. Distinct characteristics of antibody responses against factor VIII in healthy individuals and in different cohorts of hemophilia A patients. *Blood.* 2013;121(6):1039-1048.

27. Klintman J, Hillarp A, Donfield S, Berntorp E, Astermark J. Antibody formation and specificity in Bethesda-negative brother pairs with haemophilia A. *Haemophilia.* 2013;19(1):106-112.

28. Klintman J, Hillarp A, Berntorp E, Astermark J. Long-term anti-FVIII antibody response in Bethesda-negative haemophilia A patients receiving continuous replacement therapy. *Br J Haematol.* 2013;163(3):385-392.

29. Dimichele DM, Blanchette V, Berntorp E. Clinical trial design in haemophilia. *Haemophilia.* 2012;18(Suppl 4):18-23.