Safety and prolonged activity of recombinant factor VIII Fc fusion protein in hemophilia A patients

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Current factor VIII (FVIII) products display a half-life (t1/2) of ~8-12 hours, requiring frequent intravenous injections for prophylaxis and treatment of patients with hemophilia A. rFVIIIFc is a recombinant fusion protein composed of a single molecule of FVIII covalently linked to the Fc domain of human IgG1 to extend circulating rFVIII t1/2. This first-in-human study in previously treated subjects with severe hemophilia A investigated safety and pharmacokinetics of rFVIIIFc. Sixteen subjects received a single dose of rFVIII at 25 or 65 IU/kg followed by an equal dose of rFVIIIFc. Most adverse events were unrelated to study drug. None of the study subjects developed anti-rFVIIIFc antibodies or inhibitors. Across dose levels, compared with rFVIII, rFVIIIFc showed 1.54- to 1.70-fold longer elimination t1/2, 1.49- to 1.56-fold lower clearance, and 1.48- to 1.56-fold higher total systemic exposure. rFVIII and rFVIIIFc had comparable dose-dependent peak plasma concentrations and recoveries. Time to 1% FVIII activity above baseline was ~1.53- to 1.68-fold longer than rFVIII across dose levels. Each subject showed prolonged exposure to rFVIIIFc relative to rFVIII. Thus, rFVIIIFc may offer a viable therapeutic approach to achieve prolonged hemostatic protection and less frequent dosing in patients with hemophilia A. This trial was registered at www.clinicaltrials.gov as NCT01027377.

Introduction

Hemophilia A is an inherited bleeding disorder that results in frequent spontaneous and traumatic bleeding into the joints and soft tissues.1 When inadequately treated, this bleeding leads to chronic arthropathy, disability, and increased risk of death.2

Plasma-derived factor VIII and recombinant human FVIII (rFVIII) products are used for treatment (on-demand therapy) and prevention (prophylaxis therapy) of bleeding episodes. rFVIII was developed to reduce the risk of blood-borne pathogen transmission after the widespread contamination of plasma products with HIV and hepatitis viruses and to secure an adequate supply of FVIII product. However, hemostatic protection with current FVIII products is temporally limited because of a short half-life (t1/2) of ~8-12 hours, requiring prophylactic injections 3 times per week or every other day for most patients to maintain FVIII levels above 1%, a level that has been established as protective against most spontaneous bleeding episodes.3

Many studies have shown that, even at high doses, on-demand therapy is not effective in preventing arthropathy.4,5 The benefits of prophylactic therapy have been reported in numerous clinical studies4,6-16 and Manco-Johnson et al3 established that children started on primary prophylaxis after their first joint bleed had significantly fewer bleeds and less joint damage than children treated on demand.

Compared with on-demand treatment, prophylactic therapy also decreases disability, hospitalization rate, and time lost from school or work6,17 and improves quality of life for patients and their families.18 However, prophylactic therapy often requires use of central venous access devices in children and their attendant risks of infection, sepsis, and thrombosis. In addition, despite the benefits, acceptance of and compliance with prophylaxis decreases with age, in part because of inconvenience and invasiveness.19,20 Thus, a rFVIII product with a prolonged plasma t1/2 would potentially be of benefit.21 rFVIIIFc is a recombinant fusion protein composed of a single molecule of B-domain–deleted rFVIII covalently linked to the human IgG1 Fc domain. A potential advantage of Fc-fusion proteins is prolonged hemostatic protection; in addition, the Fc domain represents a natural molecule with no known inherent toxicity.22,23 Attachment to the IgG1 Fc domain permits binding to the neonatal Fc receptor (FcRn), which is expressed in many cell types, including endothelial cells. FcRn expression remains stable throughout life and is responsible for protecting IgG1 and Fc-fusion proteins from lysosomal degradation, thus prolonging the t1/2 of the protein.22,24 Other circulating proteins that lack a recycling receptor are internalized into the cells lining the vasculature by nonspecific pinocytosis and are trafficked to endosomal and lysosomal degradation pathways.24 Fc proteins interact with FcRn, resident within endosomes. Endosomes containing FcRn direct the Fc fusion proteins back to the plasma membrane, releasing them into circulation in a pH-dependent manner,25 thereby avoiding lysosomal degradation. This recycling approach has been used successfully to extend the t1/2 of therapeutic biologics; several Fc fusion-based drugs have been...
Preclinical data for rFVIIIFc indicate that FVIII can be rescued from degradation by a natural protective pathway mediated by FcRn, thus extending $t_{1/2}$. In mice and dogs with hemophilia A, terminal plasma $t_{1/2}$ for rFVIIIFc was $\sim 2$ times longer than with rFVIII. On the basis of these data, we conducted a first-in-human clinical study to investigate the safety and pharmacokinetics (PK) of a long-lasting rFVIIIFc fusion protein in subjects with hemophilia A.

**Methods**

**Study design**

This open-label, dose-escalation, multicenter phase 1/2a study in previously treated patients with severe hemophilia A investigated the safety of rFVIIIFc and its PK compared with rFVIII (antihemophilic factor VIII [recombinant], plasma/albumin-free method; Advate; Baxter Healthcare). This study was performed in accordance with the US Code of Federal Regulations and International Conference on Harmonisation Guidelines on Good Clinical Practices. Before any testing, approval from participating institutional review boards and written informed consents from all subjects were obtained in accordance with the Declaration of Helsinki. The study design was sequential; a single dose of rFVIII was administered at 25 or 65 IU/kg followed $\sim 3$ or 4 days later, respectively, by an equal dose of rFVIIIFc (Figure 1). Both drugs were injected intravenously over $\sim 10$ minutes. The 2 dose levels were expected to bracket the typical therapeutic dose ranges. Plasma FVIII activity was measured in subjects before rFVIIIFc injection, and 10 and 30 minutes, 1, 3, 6, 9, 24, 48, 72, 96, 120, and 168 hours (7 days) after injection, with additional samples at 192, 216, and 240 hours (10 days) for subjects who were dosed at 65 IU/kg rFVIIIFc. Plasma FVIII activity was measured at the same time points after rFVIII treatment, through 72 hours for the 25 IU/kg group and 96 hours for the 65 IU/kg group. Subjects were followed for 28 days after receiving rFVIIIFc for safety analyses, including testing for anti-rFVIIIFc drug antibodies and inhibitors tested by the Nijmegen-modified Bethesda assay at 14 and 28 days after injection.

**Subjects**

Male subjects were $\geq 12$ years of age with severe hemophilia A (defined as FVIII activity level $< 1\%$) and had $\geq 100$ documented prior exposure days to FVIII concentrates (plasma-derived FVIII or rFVIII). Subjects with known hypersensitivity to mouse or hamster protein, with history of inhibitor or detectable inhibitor titer at screening, who were taking any medications that could affect hemostasis or systemic immunosuppressive drugs, or who experienced an active bacterial or viral infection (other than hepatitis or HIV) within 30 days of screening were excluded. Subject’s genotype was recorded at study entry, when known.

**Treatment product**

The human rFVIIIFc and Fc transgenes were stably transfected into HEK293 cells, and the cell line was extensively tested for stability, sterility, and viral contamination to ensure safety. The purified drug product is a monomeric B-domain–deleted FVIII covalently linked through its carboxy-terminus to the N-terminus of an Fc monomer, which forms a disulfide bond with a second Fc monomer during synthesis and secretion from the cells. rFVIIIFc was purified by chromatography and nanofiltration and was fully active in one-stage (aPTT) clotting assays and chromogenic substrate assays relative to commercially available rFVIII preparations. It was supplied as a frozen liquid containing 1000 IU/2 mL of solution and formulated with L-histidine (pH 7), sodium chloride, calcium chloride, sucrose, mannitol, and Polysorbate 20. For injection, the product was diluted with 6 mL of saline solution (0.9% NaCl).

**Outcome measures**

The primary objective of the study was safety, evaluated through physical examination, reporting of treatment-emergent adverse events (AEs), development of antibodies, and laboratory monitoring over time. The secondary objectives included parameters derived from PK analyses. Laboratory assessments included prothrombin time, activated partial thromboplastin time (aPTT), international normalized ratio, levels of D-dimer, VWF Ag, standard hematology and blood chemistry tests, and urinalysis.

FVIII activity for rFVIII and rFVIIIFc was measured by the one-stage clotting (aPTT) assay on a Siemens BCS-XP analyzer with the use of commercial reagents (Dade Actin FSL) with calibration against a normal reference plasma (Precision Biologics CRYOcheck) traceable to the World Health Organization Fifth International Standard for human plasma. In addition to the aPTT assay, FVIII activity was measured by a chromogenic substrate assay with the use of a commercially available kit (Aniara BIOPHEN FVIII:C) that complies with European Pharmacopoeia recommendations. The chromogenic assay was calibrated against normal human reference plasma (Instrumentation Laboratories ORKE45), which also had a potency assigned against the human plasma standard from the World Health Organization Fifth International Standard.
Table 1. Subject demographics and disposition

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Dose groups</th>
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<tbody>
<tr>
<td></td>
<td>25 IU/kg (n = 6)</td>
</tr>
<tr>
<td>Mean age, y (minimum-maximum)</td>
<td>40.3 (23-61)</td>
</tr>
<tr>
<td>Mean weight, kg (minimum-maximum)</td>
<td>89.5 (73-105)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>White, n</td>
<td>6</td>
</tr>
<tr>
<td>Asian, n</td>
<td>0</td>
</tr>
<tr>
<td>Enrolled, n</td>
<td>6</td>
</tr>
<tr>
<td>Evaluated for safety, n</td>
<td>6</td>
</tr>
<tr>
<td>Included in PK analysis, n</td>
<td>6</td>
</tr>
<tr>
<td>Withdrawn before dosing, n</td>
<td>0</td>
</tr>
</tbody>
</table>

The lower limit of quantification for the one-stage (aPTT) and chromogenic assays was 0.5 IU/dL and 0.4 IU/dL, respectively. FVIII inhibitors were measured by the Nijmegen-modified Bethesda assay and < 0.6 BU/mL was considered negative. Anti-rFVIIIIC antibodies were assessed with a specific bridging electrochemiluminescent immunoassay that uses biotin and sulfo-tagged rFVIIIc. Assay sensitivity was determined to be 89 ng/mL with the use of an anti–human FVIII monoclonal Ab as a surrogate control.

PK analyses

A user-defined one-compartment disposition model, which automatically estimates the endogenous FVIII level and subsequent residual decay, was used in WinNonLin for analysis of the individual subject plasma FVIII activity-versus-time data after a single administration of rFVIII or rFVIIIc. Actual sampling times, doses, and duration of injection were used for calculations of parameters, including maximum activity (Cmax), t1/2, clearance (CL), volume of distribution at steady-state (Vss), area under the curve (time zero extrapolated to infinity [AUCINF]), mean residence time (MRT), and incremental recovery.

Monte Carlo simulation of rFVIIIc activity-versus-time profile

To construct FVIII activity-time profiles after dosing regimens of 25 IU/kg or 65 IU/kg, a Monte Carlo simulation was conducted with the population PK model of rFVIII and rFVIIIc. The mean estimates of model parameters (CL and volume of distribution) for both rFVIII and rFVIIIc, the interindividual variance, and the residual variability in the tested population were estimated with a one-compartment disposition model, an exponential intersubject variability model, and a proportional error model. The model construction dataset was based on the one-stage (aPTT) clotting assay activity of rFVIII and rFVIIIc from 16 subjects in this phase1/2a study. Five hundred subjects were simulated with 15 sampling points for each subject for each dosing regimen. The percentage of the population with FVIII activity ≥ 1% and 3% at different times after different dosing regimens of rFVIII or rFVIIIc was estimated.

Statistical analyses

Selected PK parameters for rFVIIIc and rFVIII were compared with an ANOVA model. PK parameters were log-transformed for these analyses, and estimated means, mean differences, and confidence intervals on the log-scale were transformed to obtain estimates for geometric means, geometric mean ratios, and confidence intervals, respectively, on the original scale. The geometric mean ratio is the geometric mean of the geometric mean ratios, and confidence intervals, respectively, on the log-scale were transformed to obtain estimates for geometric means, and estimated means, mean differences, and confidence intervals on the original scale. The geometric mean ratio is the geometric mean of the geometric mean ratios, and confidence intervals, respectively, on the log-scale were transformed to obtain estimates for geometric means.

Results

Subject disposition

Nineteen subjects were enrolled in the study (Table 1); 16 underwent PK evaluation for both rFVIII and rFVIIIc. One subject self-administered his previous product before completing the wash-out period after the dose with rFVIIIc and was thus excluded from the PK analysis but was included in the safety analysis. Three subjects were discontinued from the study before receiving either study drug: one voluntarily withdrew; a second was withdrawn by the investigator for noncompliance, and one was withdrawn at the sponsor’s request because of completion of study enrollment.

Of the subjects dosed, 6 subjects received 25 IU/kg and 10 subjects received 65 IU/kg of both rFVIII and rFVIIIc. Mean age was 34.6 years (range, 23-61 years). Genotypic identification was collected for 7 subjects, inversion of intron 22 was reported in 6 subjects, and a frameshift defect was reported in 1 subject. The genotype was unknown for 9 subjects. Thirteen subjects had hepatitis C antibodies, 4 of whom were also positive for HIV.

Safety

Forty-four treatment-emergent AEs were reported by 11 subjects (69%) during the rFVIIIc treatment and follow-up periods. This included the day of dosing with rFVIIIc through a 28-day observation period after dosing. One event, dysgeusia, occurred transiently in one subject while receiving a 65 IU/kg dose of rFVIIIc and was considered related to rFVIIIc. All other events were not related. All but 2 of these AEs were considered mild, and none led to withdrawal from the study (supplemental Table 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article).

Twenty-one of the 44 AEs were experienced by 1 subject (65 IU/kg dose group); 19 of these 21 AEs were graded as mild, and 2 were rated as moderate (headache and photophobia). Neither of the moderate AEs was deemed related to rFVIIIc by the investigator. Many of these AEs were consistent with an anxiety attack, and the subject’s constellation of signs and symptoms were reviewed by the Drug Safety Monitoring Committee.

No serious bleeding episodes were reported. No evidence of allergic reactions to injection was detected. All plasma samples tested negative for FVIII inhibitors and anti-rFVIIIc antibodies. No signs of injection site reactions were observed. No clinically meaningful changes in laboratory values were reported.

Correlation between aPTT and chromogenic activity for rFVIIIc in plasma

rFVIII and rFVIIIc activities were determined in the same assays with the use of commercially available reagents and calibration against normal human plasma standards. There was a strong correlation between the results obtained by the one-stage clotting assay and the chromogenic assay in samples that had an activity above the lower limit of quantification. Correlation coefficients
(Pearson $R^2$) of 0.94 and 0.95 were observed between the 2 assay results for 151 samples after rFVIII dosing and 185 samples after rFVIIIFc dosing, respectively. Compared with the aPTT results, the chromogenic FVIII activities were, on average, 21% higher for rFVIII and 32% higher for rFVIIIFc, not statistically significant (supplemental Figure 1). This observation led to a slightly higher estimation of exposure parameters by the chromogenic assessment for both drugs. The apparent higher FVIII recoveries by the chromogenic assay are typical for recombinant FVIII products.30-32

**Improved PK for rFVIIIFc**

The primary PK estimates were derived from one-stage (aPTT) clotting assay activity data. In subjects who received 25 or 65 IU/kg rFVIII followed by an equal dose of rFVIIIFc, the plasma FVIII activity rose sharply and reached Cmax within the first hour after dosing. The subsequent decline of the observed FVIII activity exhibited monoeponential decay characteristics until the baseline FVIII activity was reached (Figure 2). The Cmax increased proportionally to the dose, but it was comparable between equal doses of rFVIII and rFVIIIFc (Table 2). The total exposure (AUCINF) also increased proportionally to the dose. However, the AUCINF of rFVIIIFc was 1.48- and 1.56-fold greater than that of rFVIII at 25 IU/kg ($P = .002$) and 65 IU/kg ($P = .001$), respectively (Table 2).

The t1/2, MRT, CL, and Vss appeared to be independent of dose (Table 2). The geometric mean t1/2 of rFVIIIFc was 18.8 hours for both the 25-IU/kg and 65-IU/kg dose groups. This represents a 1.54- and 1.70-fold improvement over that of rFVIII (12.2 hours and 11.0 hours) at equivalent doses ($P < .001$), respectively (Table 2). The same intrasubject improvement was observed in the MRT of rFVIIIFc compared with rFVIII ($P < .001$). Consistent with improvement in the t1/2 and MRT was a corresponding reduction in intrasubject CL at doses of 25 IU/kg ($P = .002$) and 65 IU/kg ($P = .001$), respectively. There were no significant differences in Vss and incremental recovery between rFVIII and rFVIIIFc. Therefore, within each subject, rFVIIIFc showed an improved PK profile compared with rFVIII.

The improved PK profile of rFVIIIFc resulted in increased time after dosing to 1% FVIII activity, which was 1.53- and 1.68-fold longer than with rFVIII at 25 IU/kg ($P < .001$) and 65 IU/kg, respectively ($P < .001$; data not shown), suggesting a potentially longer therapeutic duration for rFVIIIFc.

**Table 2. PK parameters by one-stage clotting (aPTT) assay for rFVIIIFc and rFVIII per dose group**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>rFVIII geometric mean (95% CI)</th>
<th>rFVIIIFc geometric mean (95% CI)</th>
<th>Geometric mean ratio (95% CI)</th>
<th>rFVIII geometric mean (95% CI)</th>
<th>rFVIIIFc geometric mean (95% CI)</th>
<th>Geometric mean ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax, IU/dL</td>
<td>63.6 (59.1-68.3)</td>
<td>60.5 (53.1-69.0)</td>
<td>0.952 (0.819-1.11)</td>
<td>133 (105-168)</td>
<td>119 (103-136)</td>
<td>0.895 (0.795-1.01)</td>
</tr>
<tr>
<td>AUCinf, h × IU/dL</td>
<td>994 (723-1370)</td>
<td>1480 (1160-1880)</td>
<td>1.48 (1.26-1.76)</td>
<td>1800 (1350-2400)</td>
<td>2800 (1980-3970)</td>
<td>1.56 (1.33-1.83)</td>
</tr>
<tr>
<td>t1/2, h</td>
<td>12.2 (9.14-16.3)</td>
<td>18.8 (14.8-23.8)</td>
<td>1.54 (1.40-1.69)</td>
<td>11.0 (8.76-13.9)</td>
<td>18.8 (14.3-24.5)</td>
<td>1.70 (1.54-1.89)</td>
</tr>
<tr>
<td>MRT, h</td>
<td>17.5 (13.1-23.4)</td>
<td>27.0 (21.3-34.2)</td>
<td>1.54 (1.40-1.69)</td>
<td>15.8 (12.6-19.9)</td>
<td>27.0 (20.6-35.3)</td>
<td>1.71 (1.54-1.89)</td>
</tr>
<tr>
<td>CL, mL/h/kg</td>
<td>2.49 (1.80-3.45)</td>
<td>1.68 (1.31-2.15)</td>
<td>0.673 (0.569-0.796)</td>
<td>3.61 (2.71-4.83)</td>
<td>2.32 (1.64-3.29)</td>
<td>0.642 (0.547-0.753)</td>
</tr>
<tr>
<td>Vss, mL/kg</td>
<td>43.9 (39.3-49.0)</td>
<td>45.4 (39.3-52.5)</td>
<td>1.04 (0.947-1.13)</td>
<td>57.4 (48.3-68.3)</td>
<td>62.8 (55.2-71.5)</td>
<td>1.09 (0.976-1.22)</td>
</tr>
<tr>
<td>Incremental recovery, IU/dL per IU/kg</td>
<td>2.56 (2.36-2.78)</td>
<td>2.44 (2.12-2.81)</td>
<td>0.952 (0.819-1.11)</td>
<td>2.04 (1.61-2.59)</td>
<td>1.83 (1.59-2.10)</td>
<td>0.894 (0.795-1.01)</td>
</tr>
</tbody>
</table>

Estimated means, 95% CI for means, and mean differences were transformed to obtain estimated geometric means, 95% CI for geometric means, and geometric mean ratios, respectively.

CI indicates confidence interval.
The favorable PK profile of rFVIIIFc relative to rFVIII was also shown by FVIII activity measured in the chromogenic assay (supplemental Table 2), which was comparable to data derived from the one-stage (aPTT) clotting assays. The estimation of exposure, that is, Cmax and AUCINF, was slightly higher, however, based on the chromogenic assay than on the one-stage (aPTT) clotting assay for both rFVIII and rFVIIIFc.

Correlation between VWF and disposition of rFVIIIFc

Because most FVIII in circulation is in complex with von Willebrand factor (VWF)33 and because the genome-wide association study has identified that the genetic determinants of FVIII levels primarily depend on VWF levels,34 we examined the association between VWF and rFVIIIFc. A strong correlation was observed between VWF levels and CL and t1/2 for both rFVIIIFc and rFVIII. As shown in Figure 3, as the level of VWF increased, the CL of rFVIIIFc (P = .0016) and of rFVIII (P = .0012) decreased.

The opposite relationship was observed between the level of VWF and t1/2. As the level of VWF increased, the t1/2 of rFVIIIFc (P = .0003) and of rFVIII (P < .0001) increased. This correlation suggests that the Fc moiety of rFVIIIFc does not alter the role of VWF in protecting FVIII from clearance.

Results of simulation of rFVIIIFc activity

Adopting the PK parameters derived from this study, the Monte Carlo simulation predicts that a higher percentage of patients receiving rFVIIIFc will sustain FVIII levels > 1% or 3% compared with patients receiving equal doses of rFVIII (Table 3). For example, at a dose of 25 IU/kg, 12.2% of patients receiving rFVIII versus 71.2% of patients receiving rFVIIIFc are predicted to have FVIII trough levels > 1% on day 4; at a dose of 65 IU/kg, 11.0% of patients receiving rFVIII versus 66.4% of patients receiving rFVIIIFc are predicted to have FVIII levels > 3% on day 4.

Discussion

rFVIIIFc was well tolerated by subjects at both doses. No clinically significant changes were observed in hematology, blood chemistry, or urinalysis parameters. Most AEs were mild, unrelated to rFVIIIFc, and resolved without sequelae. No serious AEs or deaths occurred during the study, and no subjects at either dose developed neutralizing or binding antibodies to rFVIIIFc.

rFVIIIFc showed a significantly improved FVIII activity PK profile relative to rFVIII, with t1/2 and MRT across dose levels being 1.5- to 1.7-fold longer, as measured by the one-stage (aPTT) clotting assay, and 1.6- to 1.8-fold longer by the 2-stage chromogenic assay. The prolonged activity of rFVIIIFc predicts possible prolonged efficacy, allowing for a less frequent dosing regimen in the prophylactic treatment of patients with hemophilia A.

On the basis of the Monte Carlo simulation, a higher percentage of patients receiving rFVIIIFc are predicted to sustain activity levels > 1% or 3% than patients receiving equal doses of rFVIII.

Table 3. Predicted percentage of subjects achieving FVIII trough levels > 1% and 3% of normal at a specified dose regimen of rFVIII or rFVIIIFc

<table>
<thead>
<tr>
<th>Time after dosing, d</th>
<th>rFVIII 25 IU/kg</th>
<th>rFVIII 65 IU/kg</th>
<th>rFVIIIFc 25 IU/kg</th>
<th>rFVIIIFc 65 IU/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>40.0</td>
<td>67.8</td>
<td>92.6</td>
<td>99.0</td>
</tr>
<tr>
<td>4</td>
<td>12.2</td>
<td>31.0</td>
<td>71.2</td>
<td>90.0</td>
</tr>
<tr>
<td>5</td>
<td>4.20</td>
<td>13.6</td>
<td>39.4</td>
<td>71.6</td>
</tr>
<tr>
<td>7</td>
<td>0.200</td>
<td>1.40</td>
<td>7.80</td>
<td>26.4</td>
</tr>
</tbody>
</table>

Percentage of subjects with FVIII trough levels > 3%

<table>
<thead>
<tr>
<th></th>
<th>rFVIII 25 IU/kg</th>
<th>rFVIII 65 IU/kg</th>
<th>rFVIIIFc 25 IU/kg</th>
<th>rFVIIIFc 65 IU/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>10.6</td>
<td>34.6</td>
<td>62.2</td>
<td>91.0</td>
</tr>
<tr>
<td>4</td>
<td>1.60</td>
<td>11.0</td>
<td>25.4</td>
<td>66.4</td>
</tr>
<tr>
<td>5</td>
<td>0.200</td>
<td>3.20</td>
<td>7.00</td>
<td>36.2</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0.200</td>
<td>0.400</td>
<td>6.60</td>
</tr>
</tbody>
</table>
Clinical trials in larger numbers of patients are required to confirm and extend these results and to show protection from bleeding events. Despite the success of Fc fusion technology in prolonging circulating $t_{1/2}$ for a variety of protein therapeutics, rFVIII was considered too large to successfully produce dimeric Fc fusions. We thus created a monomeric Fc fusion protein, whereby a single effector molecule was covalently linked to a dimeric Fc, enabling binding to intracellular FcRn and subsequent recycling. In vitro coagulation assays show no loss of specific activity for rFVIIIFc, compared with B-domain–deleted or native FVIII, by either clotting or chromogenic assays, with the use of commercially available reagents and commonly used FVIII reference standards. In addition, these results indicate that rFVIIIFc can be reliably assayed in a clinic setting by either the one-stage clotting (aPTT) assay or the chromogenic method.

The hemophilia mouse and canine models successfully predicted the prolongation of $t_{1/2}$ of rFVIIIFc and its dependence on VWF28 (data not shown). rFVIIIFc binds with similar affinity to VWF as rFVIII. In the circulation, ~98% of FVIII is in complex with and protected by VWF, and VWF is present in a 50-fold excess compared with FVIII. It has been established that the presence of VWF decreases the clearance of FVIII and that people lacking VWF have accelerated clearance of FVIII. We have confirmed that this interaction between VWF and FVIII also occurs with rFVIIIFc. In this trial, an increase in VWF concentration in the circulation correlated with a prolonged FVIII and rFVIIIFc $t_{1/2}$. Although VWF plays a role in extending FVIII survival, it also appears to limit the degree of $t_{1/2}$ extension. Preclinical animal models of several genetically and chemically modified rFVIII constructs have shown that FVIII $t_{1/2}$ can be, at most, doubled compared with current rFVIII products. These results have been confirmed in this clinical trial. The degree to which rFVIIIFc $t_{1/2}$ can be extended may be limited by the $t_{1/2}$ (16-17 hours) of VWF, because it binds most of the circulating FVIII.

In summary, this phase 1/2a clinical trial is the first trial to show the safety and prolonged $t_{1/2}$ of rFVIIIFc in patients with severe hemophilia A. A pivotal phase 3 study is ongoing with rFVIIIFc to identify and establish effective prolonged prophylaxis dosing regimens.

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Authorship
Contribution: J.S.P. conducted research, reviewed the data, and wrote the manuscript; N.C.J., D.Q., M.V.R., G.C., X.Z., J.M., and M.B. conducted research and reviewed the data; E.L. analyzed data; J.G. designed the research and generated and analyzed data; and H.J., L.L., J.A.D., J.S., A.L., and G.F.P. designed the research, generated and analyzed data, and wrote the manuscript.


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References
21. Lilliecrap D. Improvements in factor concentrates. From www.bloodjournal.org by guest on April 2, 2017. For personal use only.


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Jerry S. Powell, Neil C. Josephson, Doris Quon, Margaret V. Ragni, Gregory Cheng, Ella Li, Haiyan Jiang, Lian Li, Jennifer A. Dumont, Jaya Goyal, Xin Zhang, Jurg Sommer, Justin McCue, Margaret Barbetti, Alvin Luk and Glenn F. Pierce