Title

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

Left running head: POWELL et al.

Short Title: PROLONGED rFVIIIFc ACTIVITY IN HEMOPHILIA A

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Abstract

Current factor VIII (FVIII) products display a half-life ($t_{1/2}$) of approximately 8-12 hours, requiring frequent intravenous injections for prophylaxis and treatment of hemophilia A patients. 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 half-life. 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 Advate® 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, as compared with Advate, rFVIIIFc showed 1.5 to 1.7-fold longer elimination $t_{1/2}$, 1.49 to 1.56-fold lower clearance, and 1.48 to 1.56-fold higher total systemic exposure. Advate and rFVIIIFc had comparable dose-dependent peak plasma concentrations and recoveries. Time to 1% FVIII activity above baseline was approximately 1.53 to 1.68-fold longer than Advate across dose levels. Each subject demonstrated prolonged exposure to rFVIIIFc relative to Advate. Thus, rFVIIIFc may offer a viable therapeutic approach to achieve prolonged hemostatic protection and less frequent dosing in patients with hemophilia A. Trial registered at www.clinicaltrials.gov (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 FVIII (pdFVIII) and recombinant human FVIII (rFVIII) products are utilized for treatment (on-demand therapy) and prevention (prophylaxis therapy) of bleeding episodes. rFVIII was developed to reduce the risk of blood-borne pathogen transmission following 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 due to a short half-life (t\(_{1/2}\)) of approximately 8-12 hours, requiring prophylactic injections three times per week or every other day for most patients in order 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 demonstrated in numerous clinical studies\(^4\)\(^-\)\(^16\) and Manco-Johnson et al 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.\(^3\)

Compared to on-demand treatment, prophylactic therapy also decreases disability, hospitalization rate, and time lost from school or work;\(^6,17\) 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.\textsuperscript{19,20} Thus, a rFVIII product with a prolonged plasma t\textsubscript{1/2} would potentially be of benefit.\textsuperscript{21} rFVIIIFc is a recombinant fusion protein composed of a single molecule of B-domain deleted rFVIII covalently linked to the human IgG\textsubscript{1} 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.\textsuperscript{22,23} Attachment to the IgG\textsubscript{1} 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 IgG\textsubscript{1} and Fc-fusion proteins from lysosomal degradation, thus prolonging the t\textsubscript{1/2} of the protein.\textsuperscript{22,24} Other circulating proteins, that lack a recycling receptor, are internalized into the cells lining the vasculature via nonspecific pinocytosis and are trafficked to endosomal and lysosomal degradation pathways.\textsuperscript{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,\textsuperscript{25} thereby avoiding lysosomal degradation. This recycling approach has been used successfully to extend the t\textsubscript{1/2} of therapeutic biologics; a number of Fc fusion-based drugs have been approved for clinical use (eg etanercept, romiplostim) and others are in development.\textsuperscript{26,27} Preclinical data for rFVIIIFc indicate that FVIII can be rescued from degradation by a natural protective pathway mediated by FcRn, thus extending t\textsubscript{1/2}. In Hemophilia A mice and dogs, terminal plasma t\textsubscript{1/2} for rFVIIIFc was approximately 2 times longer than with
rFVIII. Based on these data, we conducted a first-in-human clinical study to investigate the safety and 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 rFVIIIIFc and its pharmacokinetics (PK) compared with Advate® (antihemophilic factor [recombinant], plasma/albunin-free method, octocog alfa, 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. Prior to 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 Advate was administered at 25 or 65 IU/kg followed approximately 3 or 4 days later, respectively, by an equal dose of rFVIIIIFc (Figure 1). Both drugs were injected intravenously over approximately 10 minutes. The two dose levels were expected to bracket the typical therapeutic dose ranges. Plasma FVIII activity was measured in subjects before rFVIIIIFc 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 dosed at 65 IU/kg of rFVIIIIFc. Plasma FVIII activity was measured at the same time points after Advate 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 rFVIIIIFc for safety analyses, including testing for anti-rFVIIIIFc drug antibodies and inhibitors tested by the Nijmegen-modified Bethesda assay at 14 and 28 days post-injection.
Subjects

Male subjects were at least 12 years of age with severe hemophilia A (defined as FVIII activity level < 1%) and had at least 100 documented prior exposure days to FVIII concentrates (pdFVIII or rFVIII). Subjects with known hypersensitivity to mouse or hamster protein, history of inhibitor or detectable inhibitor titer at screening, or 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 rFVIIIIfc 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 composed of 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. rFVIIIIfc was purified by chromatography and nanofiltration, and was fully active in one-stage and chromogenic clotting assays relative to commercially available rFVIII preparations. It was supplied as a frozen liquid containing 1000 IU per 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 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, von Willebrand factor (VWF) antigen, standard hematology and blood chemistry tests, and urinalysis.

FVIII activity for Advate and rFVIIIFc was measured by the one-stage clotting (aPTT) assay on a Siemens BCS-XP analyzer using commercial reagents (Dade Actin FSL) with calibration against a normal reference plasma (Precision Biologics CRYOcheck™) traceable to the World Health Organization (WHO) 5th International Standard (IS) for human plasma. In addition to the aPTT assay, FVIII activity was measured by a chromogenic substrate assay29 using 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 WHO 5th IS human plasma standard.

The lower limit of quantification (LLOQ) for the one-stage 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 less than 0.6 BU/mL was considered negative. Anti-rFVIIIFc antibodies were assessed using a specific bridging electrochemiluminescent immunoassay which uses biotin and sulfo-tagged rFVIIIFc.
Assay sensitivity was determined to be 89 ng/mL using an anti-human FVIII monoclonal antibody as a surrogate control.

Pharmacokinetic Analyses

A user-defined one-compartment disposition model, which automatically estimates the endogenous FVIII level and subsequent residual decay, was utilized in WinNonLin for analysis of the individual subject plasma FVIII activity-versus-time data following a single administration of Advate or rFVIIIFc. Actual sampling times, doses, and duration of injection were used for calculations of parameters including maximum activity (C$_{max}$), t$_{1/2}$, clearance (CL), volume of distribution at steady-state (V$_{ss}$), area under the curve (time zero extrapolated to infinity [AUC$_{INF}$]), mean residence time (MRT), and incremental recovery.

Monte Carlo Simulation of rFVIIIFc Activity-Versus-Time Profile

To construct FVIII activity-time profiles following dosing regimens of 25 IU/kg or 65 IU/kg, a Monte Carlo simulation was conducted using the population PK model of Advate and rFVIIIFc. The mean estimates of model parameters (CL and volume of distribution) for both Advate and rFVIIIFc, the inter-individual variance, and the residual variability in the tested population were estimated using a one-compartment disposition model, an exponential inter-subject variability model, and a proportional error model. The model construction dataset was based on the one-stage (aPTT) clotting assay activity of Advate and rFVIIIFc 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 above or equal to 1% and 3% at different times following different dosing regimens of Advate or rFVIIIFc was estimated.
Statistical Analyses

Selected PK parameters for rFVIIIFc and Advate were compared using an analysis of variance 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 (GMR), and confidence intervals, respectively, on the original scale. The GMR is the geometric mean of the intra-subject ratio of the rFVIIIFc PK parameter value to the Advate PK parameter value.
Results

Subject Disposition

Nineteen subjects were enrolled in the study (Table 1); 16 underwent PK evaluation for both Advate and rFVIIIfc. One subject self-administered his previous product prior to completing the wash-out period following the dose with Advate 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 non-compliance; and one was withdrawn at the Sponsor’s request due to completion of study enrollment.

Of the subjects dosed, six subjects received 25 IU/kg and 10 subjects received 65 IU/kg of both Advate and rFVIIIfc. Mean age was 34.6 years (23 to 61 years). Genotypic identification was collected for seven subjects; inversion of intron 22 was reported in six subjects; and a frame-shift defect was reported in one subject. The genotype was unknown for nine subjects. Thirteen subjects had hepatitis C antibodies, four of whom were also positive for HIV.

Safety

Forty-four treatment-emergent AEs were reported by 11 (69%) subjects during the rFVIIIfc treatment and follow-up periods. This included the day of dosing with rFVIIIfc through a 28-day post-dosing observation period. One event, dysgeusia, occurred transiently in one subject while receiving a 65 IU/kg dose of rFVIIIfc and was considered related to rFVIIIfc. All other events were not related. All but two of these
AEs were considered mild and none led to withdrawal from the study (Supplemental Table 1).

Twenty-one of the 44 adverse events were experienced by one subject (65 IU/kg dose group); 19 of these 21 AEs were graded as mild, and two were rated as moderate (headache and photophobia). Neither of the moderate AEs was deemed related to rFVIIIIFc 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-rFVIIIIFc 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 rFVIIIIFc in Plasma**

Advate and rFVIIIIFc activities were determined in the same assays using 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 LLOQ. Correlation coefficients (Pearson R²) of 0.94 and 0.95 were observed between the two assay results for 151 samples following Advate dosing and 185 samples following rFVIIIIFc dosing, respectively. Compared to the aPTT results, the chromogenic FVIII activities were, on average, 21% higher for Advate and 32% higher for rFVIIIIFc, 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 tested in clinical assays, and are in agreement with most other marketed FVIII products.30-32

Improved Pharmacokinetics for rFVIIIIFc

The primary PK estimates were derived from one-stage (aPTT) clotting assay activity data. In subjects who received 25 or 65 IU/kg of Advate followed by an equal dose of rFVIIIIFc, the plasma FVIII activity rose sharply and reached $C_{\text{max}}$ within the first hour following dosing. The subsequent decline of the observed FVIII activity exhibited monoexponential decay characteristics until the baseline FVIII activity was reached (Figure 2). The $C_{\text{max}}$ increased proportionally to the dose, but was comparable between equal doses of Advate and rFVIIIIFc (Table 2). The total exposure ($\text{AUC}_{\text{INF}}$) also increased proportionally to the dose. However, the $\text{AUC}_{\text{INF}}$ of rFVIIIIFc was 1.48 and 1.56-fold greater than that of Advate at 25 IU/kg ($p=0.002$) and 65 IU/kg ($p<0.001$), respectively (Table 2).

The $t_{1/2}$, MRT, CL, and $V_{ss}$ appeared to be independent of dose (Table 2). The geometric mean $t_{1/2}$ of rFVIIIIFc 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 Advate (12.2 hours and 11.0 hours) at equivalent doses ($p<0.001$), respectively (Table 2). The same intra-subject improvement was observed in the MRT of rFVIIIIFc compared with Advate ($p<0.001$). Consistent with improvement in the $t_{1/2}$ and MRT was a corresponding reduction in intra-subject CL at doses of 25 IU/kg ($p=0.002$) and 65 IU/kg ($p<0.001$), respectively. There were no significant differences in $V_{ss}$ and incremental recovery between Advate and rFVIIIIFc. Therefore, within each subject, rFVIIIIFc demonstrated an improved PK profile compared with Advate.
The improved PK profile of rFVIIIFc resulted in increased time post-dosing to 1% FVIII activity which was 1.53 and 1.68-fold longer respectively, than with Advate at 25 IU/kg (p<0.001) and 65 IU/kg (p<0.001) (data not shown), suggesting a potentially longer therapeutic duration for rFVIIIFc.

The favorable PK profile of rFVIIIFc relative to Advate was also demonstrated 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, ie, Cmax and AUCINF, was slightly higher, however, based on the chromogenic assay than on the one-stage (aPTT) clotting assay for both Advate and rFVIIIFc.

Correlation Between von Willebrand Factor and Disposition of rFVIIIFc
Because the majority of FVIII in circulation is in complex with VWF and because the genome-wide association study has identified that the genetic determinants of FVIII levels are primarily dependent on VWF levels, 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 Advate. As shown in Figure 3, as the level of VWF increased, the CL of rFVIIIFc (p=0.0016) and of Advate (p=0.0012) 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=0.0003) and of Advate (p<0.0001) 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 above 1% or 3% as compared with patients receiving equal doses of Advate (Table 3). For example, at a dose of 25 IU/kg, 12.2% of Advate patients versus 71.2% of rFVIIIFc patients are predicted to have FVIII trough levels above 1% on Day 4; at a dose of 65 IU/kg, 11.0% of Advate patients versus 66.4% of rFVIIIFc patients are predicted to have FVIII levels above 3% on Day 4.
Discussion

rFVIIIFc was well tolerated by subjects at both doses. There were no clinically significant changes observed in hematology, blood chemistry, or urinalysis parameters. The majority of 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 demonstrated a significantly improved FVIII activity PK profile relative to Advate, with t\(\frac{1}{2}\) 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 two-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.

Based on the Monte Carlo simulation, a higher percentage of patients receiving rFVIIIFc are predicted to sustain activity levels above 1% or 3% as compared with patients receiving equal doses of Advate (Table 3). Clinical trials in larger numbers of patients are required to confirm and extend these results and to demonstrate protection from bleeding events.

Despite the success of Fc fusion technology in prolonging circulating t\(\frac{1}{2}\) 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 covalent linked to a dimeric Fc, enabling binding to intracellular FcRn and subsequent recycling.\(^\text{22,23}\) In vitro coagulation assays demonstrate no loss of specific activity for rFVIIIFc, compared to B-domain deleted or native FVIII, by either clotting or
chromogenic assays, using 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 assay or the chromogenic method. The hemophilia mouse and canine models successfully predicted the prolongation of half-life of rFVIIIFc and its dependence on VWF (data not shown). rFVIIIFc binds with similar affinity to VWF as rFVIII. In the circulation, approximately 98% of FVIII is in complex with and protected by VWF and VWF is present in a 50 fold excess compared to FVIII.

It has been established that the presence of VWF decreases the clearance rate of FVIII and that people lacking VWF have accelerated clearance rates for 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 half-life. While VWF plays a role in extending FVIII survival, it also appears to limit the degree of half-life extension. Preclinical animal models of several genetically and chemically modified rFVIII constructs have shown that FVIII half-life can be, at most, doubled compared with current rFVIII products. These results have been confirmed in this clinical trial. The degree to which rFVIIIFc half-life can be extended may be limited by the half-life (16 to 17 hours) of VWF, as it binds the majority of circulating FVIII.

In summary, this Phase 1/2a clinical trial is the first trial to demonstrate 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.
Acknowledgements

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Authorship Contributions

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, generated and analyzed data, 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.

Disclosure of Conflicts of Interest

D.Q., M.V.R., and G.C. received research support from Biogen Idec, which conducted the study.
References


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Table. 1. Subject Demographics and Disposition

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>25 IU/kg n = 6</th>
<th>65 IU/kg n = 10</th>
<th>Total N = 16</th>
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<tr>
<td>Mean age years min, max</td>
<td>40.3 (23 – 61)</td>
<td>31.1 (23 – 42)</td>
<td>34.6 (23 – 61)</td>
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<td>Mean weight (kg) min, max</td>
<td>89.5 (73 – 105)</td>
<td>78.7 (54 – 111)</td>
<td>82.7 (54 – 111)</td>
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<tr>
<td>Number of subjects</td>
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<td>9</td>
<td>15</td>
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<tr>
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<td>White</td>
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<td>9</td>
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<tr>
<td>Evaluated for safety</td>
<td>6</td>
<td>10</td>
<td>16</td>
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<tr>
<td>Included in PK analysis</td>
<td>6</td>
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<tr>
<td>Withdrawn prior to dosing</td>
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PK = pharmacokinetic
Table 2. PK Parameters by One-Stage (aPTT) Assay for rFVIIIFc and Advate Per Dose Group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dose: 25 IU/kg (N=6)</th>
<th></th>
<th>Dose: 65 IU/kg (N=9)</th>
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<td></td>
<td>Advate Geom. Mean [95% CI]</td>
<td>rFVIIIFc Geom. Mean [95% CI]</td>
<td>Geom. Mean Ratio [95% CI] (p-value)</td>
<td>Advate Geom. Mean [95% CI]</td>
</tr>
<tr>
<td>C_max (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] (p = 0.440)</td>
<td>133 [105, 168]</td>
</tr>
<tr>
<td>AUC_{INF} (hr*IU/dL)</td>
<td>994 [723, 1370]</td>
<td>1480 [1160, 1880]</td>
<td>1.48 [1.26,1.76] (p = 0.002)</td>
<td>1800 [1350, 2400]</td>
</tr>
<tr>
<td>t_{1/2} (hr)</td>
<td>12.2 [9.14, 16.3]</td>
<td>18.8 [14.8, 23.8]</td>
<td>1.54 [1.40,1.69] (p &lt; 0.001)</td>
<td>11.0 [8.76, 13.9]</td>
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<td>MRT (hr)</td>
<td>17.5 [13.1, 23.4]</td>
<td>27.0 [21.3, 34.2]</td>
<td>1.54 [1.40,1.69] (p &lt; 0.001)</td>
<td>15.8 [12.6, 19.9]</td>
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<td>CL (mL/hour/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] (p = 0.002)</td>
<td>3.61 [2.71, 4.83]</td>
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<td>V_{ss} (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] (p = 0.357)</td>
<td>57.4 [48.3, 68.3]</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] (p = 0.444)</td>
<td>2.04 [1.61, 2.59]</td>
</tr>
</tbody>
</table>

CI = Confidence Interval; Geom. Mean = Geometric Mean. 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.
Table 3. Predicted Percentage of Subjects Achieving FVIII Trough Levels Above 1% and 3% of Normal at a Specified Dose Regimen of Advate or rFVIIIFc

<table>
<thead>
<tr>
<th>Timepoint following dosing (Day)</th>
<th>Advate</th>
<th></th>
<th>rFVIIIFc</th>
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<tbody>
<tr>
<td></td>
<td>25 IU/kg</td>
<td>65 IU/kg</td>
<td>25 IU/kg</td>
<td>65 IU/kg</td>
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Figure Legends

Figure 1. Study Design
The Phase 1/2a study was a dose-escalation, sequential design to evaluate the safety and PK of rFVIIIFc compared with Advate after a single intravenous dose of either 25 IU/kg (low dose cohort) or 65 IU/kg (high dose cohort).

Figure 2. Group Mean Plasma FVIII Activity Pharmacokinetic Profiles for Low-Dose and High-Dose Cohorts
The plasma FVIII activity (one stage aPTT assay) versus time curve after a single intravenous injection of rFVIIIFc or Advate are shown for (A) 25 IU/kg (low-dose cohort, n=6) and (B) 65 IU/kg (high dose cohort, n=10 [Advate]; n=9 [rFVIIIFc]). Results presented are group mean ± standard error of mean (SEM).

Figure 3. Effect of VWF Antigen Levels on Cl and t1/2 of FVIII Activity after Injection of Advate or rFVIIIFc
Correlation between VWF antigen levels and (A) the weight-adjusted Cl of Advate (R²=0.5415 and p=0.0012) and rFVIIIFc (R²=0.5492 and p=0.0016) and (B) the t1/2 of Advate (R²=0.7923 and p<0.0001) and rFVIIIFc (R²= 0.6403 and p=0.0003). Each dot represents an individual subject.
Figure 1.

Cohort A: low dose

Cohort B: high dose

Dosing & PK

Dosing & PK

Screening

Wash out

Advate®
(25 IU/kg)

rFVIII:Fc
(25 IU/kg)

Follow up

28 days

3 days

7 days

21 days

Dose escalation

28 days

4 days

10 days

18 days
Figure 2.

(A) Plasma FVIII Activity (IU/dL) vs. Time After Start of Infusion (Hr)

- Advate (25 IU/kg)
- rFVIIIFc (25 IU/kg)

(B) Plasma FVIII Activity (IU/dL) vs. Time After Start of Infusion (Hr)

- Advate (65 IU/kg)
- rFVIIIFc (65 IU/kg)
Figure 3.

(A) CL (mL/hr/kg) vs. VWF Antigen (%)
- rFVIIIFc
- Advate

R² = 0.5415, p = 0.0012
R² = 0.5492, p = 0.0016

(B) T₁/₂ (h) vs. VWF Antigen (%)
- rFVIIIFc
- Advate

R² = 0.6403, p = 0.0003
R² = 0.7923, p < 0.0001
Safety and prolonged activity of recombinant factor VIII Fc fusion protein in hemophilia A patients

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