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ORIGINAL ARTICLE

Recombinant factor VIII Fc fusion protein for the prevention and treatment of bleeding in children with severe hemophilia A

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Summary. *Background:* Prophylactic factor replacement, which prevents hemarthroses and thereby reduces the musculoskeletal disease burden in children with hemophilia A, requires frequent intravenous infusions (three to four times weekly). *Objective:* Kids A-LONG was a phase 3 open-label study evaluating the safety, efficacy and pharmacokinetics of a longer-acting factor, recombinant factor VIII Fc fusion protein (rFVIIIFc), in previously treated children with severe hemophilia A (endogenous FVIII level of < 1 IU dL^{-1} [< 1%]). *Methods:* The study enrolled 71 subjects. The starting rFVIIIFc regimen was twice-weekly prophylaxis (Day 1, 25 IU kg^{-1}; Day 4, 50 IU kg^{-1}); dose (≤ 80 IU kg^{-1}) and dosing interval (≥ 2 days) were adjusted as needed. A subset of subjects had sequential pharmacokinetic evaluations of FVIII and rFVIIIFc. The primary endpoint was development of inhibitors (neutralizing antibodies). Secondary endpoints included pharmacokinetics, annualized bleeding rate (ABR), and number of infusions required to control a bleed. *Results:* No subject developed an inhibitor to rFVIIIFc. Adverse events were typical of a pediatric hemophilic population. The rFVIIIFc half-life was prolonged relative to that of FVIII, consistent with observations in adults and adolescents. The median ABR was 1.96 overall, and 0.00 for spontaneous bleeds; 46.4% of subjects reported no bleeding episodes on study. Ninety-three per cent of bleeding episodes were controlled with one to two infusions. The median average weekly rFVIIIFc prophylactic dose was 88.11 IU kg^{-1}. At study end, 62 of 69 subjects (90%) were infusing twice weekly. Among subjects who had been previously receiving FVIII prophylaxis, 74% reduced their dosing frequency with rFVIIIFc. *Conclusion:* Twice-weekly infusions with rFVIIIFc were well tolerated and yielded low bleeding rates in children with severe hemophilia A.

Keywords: children; coagulation factor VIII; hemophilia A; prophylaxis; recombinant fusion proteins.

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Introduction

In patients with severe hemophilia A, recurrent spontaneous bleeding into joints and muscles is common, and results in chronic pain and impaired joint function [1,2]. Early prophylactic replacement of coagulation factor VIII (FVIII) has been shown to improve long-term clinical outcomes [1,3,4]. However, the frequent intravenous infusions (three to four times weekly) required to maintain protective FVIII levels above 1 IU dL^{-1} (1%) [3,5] can constitute a barrier to the adoption of prophylaxis [6–8], particularly for children with challenging venous access and in whom more frequent infusions may be required, owing to a shorter FVIII half-life than that in adults [9]. A FVIII molecule with a prolonged circulation time has the potential to improve the adoption of and adherence to prophylaxis, and may positively impact therapeutic outcomes [8].

Recombinant FVIII Fc fusion protein (rFVIIIFc) is composed of a single molecule of recombinant FVIII

(rFVIII) covalently fused to the dimeric Fc domain of IgG_1 [10]; the protein binds the neonatal Fc receptor, and utilizes the IgG recycling pathway to extend plasma half-life [11]. In the phase 3 A-LONG study [12] of previously treated adults and adolescents with hemophilia A, rFVIIIFc had an approximately 1.5-fold longer half-life than rFVIII, and was shown to be safe and efficacious for prophylaxis. Here, we report the results of a multicenter, open-label phase 3 study evaluating the safety, efficacy and pharmacokinetics (PK) of rFVIIIFc in previously treated children aged < 12 years with severe hemophilia A.

Materials and methods

Study population

This was a phase 3 open-label multicenter study of rFVIIIFc in pediatric patients with severe hemophilia A. The protocol was approved by individual institutional review boards; the study was conducted in accordance with the International Conference on Harmonization guidelines for Good Clinical Practice, and registered with ClinicalTrials.gov (NCT01458106). All subjects' guardians provided written informed consent; if appropriate, subjects also provided assent.

Children aged < 12 years with severe hemophilia A (endogenous FVIII activity of < 1 IU dL^{-1} [< 1%]) or a documented genotype known to produce severe hemophilia A [13,14] were eligible for inclusion in the study. In accordance with regulatory guidance [15], and to allow for evaluation of the antigenicity of rFVIIIFc, the study was limited to previously treated patients. Patients were considered to have been previously treated if they had received treatment with any recombinant or plasma-derived FVIII product for at least 50 exposure days (EDs; defined as a 24-h period during which replacement factor was administered one or more times). Patients with a history of or currently detectable inhibitor (i.e. neutralizing antibody activity at screening of ≥ 0.6 BU mL^{-1} determined with the Nijmegen-modified Bethesda assay), history of anaphylaxis associated with any FVIII or intravenous immunoglobulin administration, or other coagulation disorders in addition to hemophilia A, were excluded. Additional criteria are specified in Table S1.

Study design

The study had a single prophylactic treatment arm with twice-weekly intravenous infusions of rFVIIIFc (25 IU kg^{-1} on Day 1 and 50 IU kg^{-1} on Day 4) (Fig. 1). Adjustments in dose (to a maximum of 80 IU kg^{-1}) and interval (to a minimum of every 2 days) were allowed, based on a subject's available PK data and observed bleeding patterns (Fig. S1).

As per regulatory guidance [15], a subset of subjects (a minimum of 12 in each cohort) had comparative PK assessments with their prestudy FVIII product and rFVIIIFc prior to initiation of rFVIIIFc prophylaxis. For PK assessments, a washout period of at least 72 h with no FVIII treatment was required prior to administration of either prestudy FVIII (50 IU kg^{-1}, rounded up to the nearest 250-IU increment, with sampling up to 48 h) or administration of rFVIIIFc (50 IU kg^{-1}, with sampling up to 72 h); all samples collected were analyzed for FVIII activity in plasma at a central laboratory.

The study sample size was based on clinical considerations and regulatory requirements [15], with a target enrollment of ~ 68 subjects (~ 34 aged < 6 years, and ~ 34 aged 6 years to < 12 years); taking into account a 25% drop-out rate, this would ensure that at least 25 subjects per age cohort had valid inhibitor test results following at least 50 rFVIIIFc EDs. The study was terminated when at least 12 subjects in each age group had adequate PK data, and ≥ 50 subjects had valid inhibitor test results from testing at 50 EDs or more.

The dose and dosing interval compliance rate of each subject with the prescribed prophylactic dosing regimen was calculated on both a per-injection basis and a per-subject basis. Compliance evaluations considered the nominal dose taken per infusion as compared with the nominal dose prescribed, and the actual day of treatment as compared with the prescribed day of treatment. A subject with $\geq 80\%$ of doses taken within 80–125% of the prescribed dose and $\geq 80\%$ of doses taken within ± 24 h of the prescribed day was considered to be 'dose compliant' and 'dosing interval compliant', respectively.

Outcome measures

The primary endpoint was the development of inhibitors (identified by a Nijmegen-modified Bethesda assay titer of ≥ 0.6 BU mL^{-1}, and confirmed in two separate samples drawn approximately 2–4 weeks apart), with testing being conducted at each clinic visit prior to dosing; all samples were analyzed at a central laboratory. A washout period of at least 48 h was required prior to inhibitor testing (washout was confirmed from a trough sample at baseline); samples were heat-inactivated prior to inhibitor testing to inactivate any residual rFVIIIFc that may have been present. Testing for inhibitors was conducted within the first 10–15 EDs to rFVIIIFc and at all scheduled visits. If the 10–15 ED testing did not occur at a scheduled study visit, an additional visit was scheduled to complete the testing. If inhibitor development was suspected at any time during the study (for example, if the expected plasma FVIII activity levels were not attained or if the bleeding episode was not controlled with an expected dose), the subject was tested for inhibitors by a central laboratory.
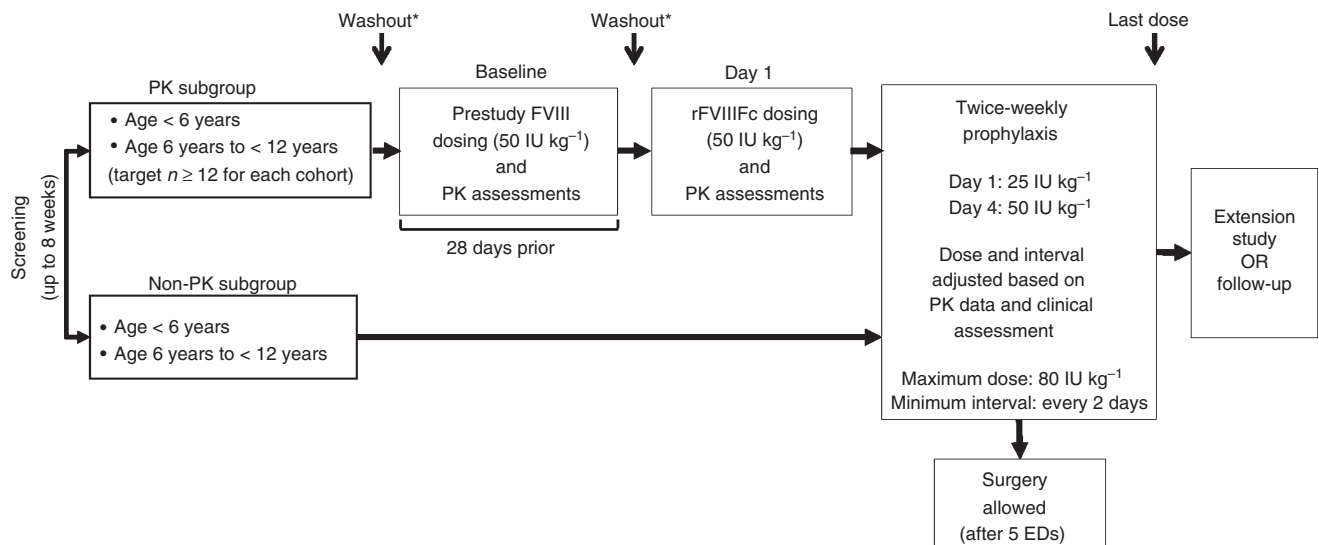
Fig. 1. Study design. Kids A-LONG was an open-label, multicenter evaluation of recombinant FVIII Fc fusion protein (rFVIIIFc) in pediatric previously treated patients with severe hemophilia A. The study enrolled 71 subjects into two age cohorts (< 6 years of age; 6 years to < 12 years of age). A sufficient number of subjects (at least 12 from each age cohort) were to be enrolled into a comparative pharmacokinetic (PK) subgroup for the sequential evaluation of PK profiles for prestudy FVIII and rFVIIIFc (FVIII evaluation was to occur at least 28 days prior to the first rFVIIIFc dose). The time required for screening and PK assessments was approximately 8–12 weeks. All subjects began twice-weekly prophylactic treatment with rFVIIIFc, with a starting regimen of 25 IU kg^{-1} on Day 1 and 50 IU kg^{-1} on Day 4. If a subject had at least five exposure days (EDs) of rFVIIIFc without safety concerns, surgery was allowed. The duration of study participation per subject was ~ 28 weeks for the treatment and follow-up periods, after which subjects had the option to enter into an extension study. *A washout period of at least 72 h with no FVIII treatment was required prior to administration of prestudy FVIII and prior to rFVIIIFc for PK assessments.

Secondary endpoints included FVIII activity (PK) measurements, annualized bleeding rate (ABR) by type (spontaneous or traumatic) and location of bleeding, rFVIIIFc dose administered for treatment of a bleeding episode, and subject's rating of the response to rFVIIIFc for the treatment of bleeding. The protocol for the treatment of a bleeding episode is detailed in Fig. S2. Clinical and laboratory safety assessments were also performed, and the development of anti-rFVIIIFc binding antibodies was assessed at baseline and at each clinic visit by means of an electrochemiluminescence assay [16] for rFVIIIFc-binding antibodies.

Analytical methods

Statistical analyses were primarily descriptive in nature, with data being summarized overall and by age cohort. Safety analyses were performed with data from all subjects (i.e. those who received at least one dose of a prestudy FVIII treatment for the purpose of evaluating PK, or at least one dose of rFVIIIFc); efficacy analyses included data only from subjects who received at least one dose of rFVIIIFc. Efficacy data from the last 3 months on study were analyzed to account for dosage adjustments that may have occurred early in the study, as well as any underlying physiologic changes related to the adjustment to a new treatment regimen (e.g. changing from episodic to prophylactic treatment) that may have contributed to a higher initial rate of bleeding.

Efficacy outcomes were evaluated by the use of descriptive statistics (median and interquartile range [IQR]). For prestudy versus on-study dosing analyses, the prestudy regimen evaluated was the most recent regimen that the subject had received prior to the study. Actual sampling times, actual doses of rFVIIIFc, nominal doses of prestudy FVIII and actual injection durations were used for PK analyses. The use of nominal doses in analyses of prestudy FVIII was necessary because of geographic differences in the availability of prestudy product information (for example, in the USA, actual vial strength information for commercial FVIII products was available, whereas only nominal vial strength information was available outside the USA).

PK parameters based on FVIII activity as measured with the one-stage clotting assay are presented as arithmetic means and 95% confidence intervals (CIs); comparative intrasubject ratios (rFVIIIFc/prestudy FVIII) of the PK parameters are expressed as medians and IQRs.

Results

Study population

A total of 71 male subjects (< 6 years of age, $n = 36$; 6 years to < 12 years of age, $n = 35$) were enrolled at 23 investigational sites in eight countries between 9 October 2012 and 5 December 2013; 67 subjects (94.4%) completed the study (Fig. 2). Subjects' demographics and
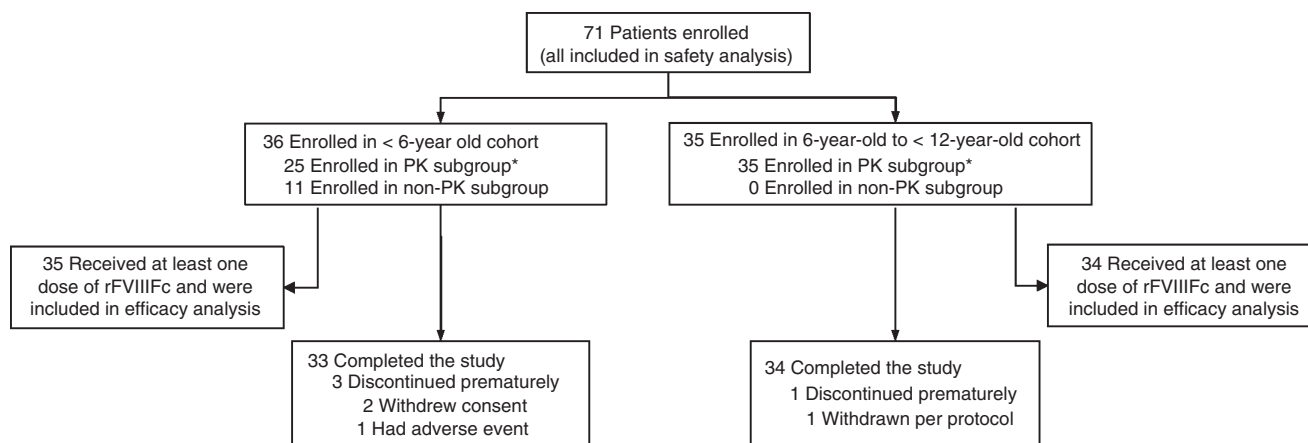
Fig. 2. Subject disposition. Patients were assigned to the appropriate age cohort (< 6 years of age or 6 years to < 12 years of age) at the time of screening; the assigned cohort was used in all analyses unless otherwise stated. All enrolled patients were included in the safety analysis; two subjects received prestudy FVIII for the purpose of evaluating pharmacokinetics (PK), but were not treated with recombinant FVIII Fc fusion protein (rFVIIIFc), and were therefore not included in the efficacy analysis. Sixty patients were enrolled in the PK subgroup and underwent PK assessments with both prestudy FVIII and rFVIIIFc prior to the initiation of an rFVIIIFc prophylaxis regimen. Of these, 51 subjects (< 6 years of age, *n* = 23; 6 years to < 12 years of age, *n* = 28) had complete evaluable PK profiles for both prestudy FVIII and rFVIIIFc. Four subjects did not complete the study: two withdrew consent, one was withdrawn per protocol (washout attempts prior to rFVIIIFc PK assessment were unsuccessful), and one had an adverse event (infection of a central venous catheter) that led to study discontinuation prior to receipt of treatment with rFVIIIFc. *Of the 60 subjects in the PK subgroup, five were exempted from PK assessment with prestudy FVIII at screening because they had historical data (i.e. within 14 months of screening) from a prior PK assessment with FVIII.

baseline characteristics were representative of the global pediatric hemophilia A population (Table 1). As expected for a pediatric population, no enrolled subjects were positive for human immunodeficiency virus or hepatitis C virus infection. A family history of inhibitors, which was not prohibited per protocol, was reported in six study subjects (8.5%), and a large proportion of subjects had genotypes known to be associated with inhibitor development [13,14] (e.g. intron 22 inversions in 26 subjects [36.6%] and nonsense mutations in nine subjects [12.7%]). The majority of subjects (88.7%) were receiving FVIII prophylaxis at study entry, and ~ 75% of these subjects were infusing three or more times per week.

Of the 71 enrolled subjects, 69 were exposed to rFVIIIFc during the study. The median time on study was 26.3 weeks, and a total of 61 subjects (88.4%) had ≥ 50 rFVIIIFc EDs on study. The majority (69.6%) of subjects were adherent to their prescribed dose and interval, although the dose-and-interval compliance rate was lower in the younger cohort (51.4%) than in the older cohort (88.2%). This was likely attributable to the protocol requirement of 'rounding up' to the nearest dose increment in younger subjects with low body weights, as shown by the fact that, whereas dosing interval compliance was similar in the younger and older cohorts (88.6% and 97.1%, respectively), compliance with the prescribed dose was not (60.0% and 91.2%, respectively).

Safety

No subjects developed an inhibitor to rFVIIIFc. The estimated inhibitor incidence rate was 0.00% (95% CI 0.00–5.06%) overall, and 0.00% (95% CI 0.00–5.87%) among 61 subjects with at least 50 rFVIIIFc EDs. No subject had a positive test result for anti-rFVIIIFc binding antibodies that emerged with rFVIIIFc treatment. Anti-rFVIIIFc binding antibodies (i.e. anti-drug antibodies) were detected at baseline in seven subjects (< 6 years of age, *n* = 4; 6 years to < 12 years of age, *n* = 3); in all cases, the positive anti-rFVIIIFc binding antibody results were obtained prior to the first dose of rFVIIIFc. In these seven subjects, the positive anti-rFVIIIFc binding antibody result was followed by a negative result during the course of the study (four subjects), or fluctuated but remained positive through all study visits (three subjects). Importantly, none of the anti-rFVIIIFc binding antibodies detected was directed against the Fc portion of rFVIIIFc. These anti-rFVIIIFc binding antibodies had no discernable impact on PK assessments, adverse events (AEs), or bleeding rates. Further evaluation of the potential impact of anti-rFVIIIFc binding antibodies on PK and FVIII activity was not feasible, owing to the small number subjects with positive test results.

rFVIIIFc was generally well tolerated, with a pattern of AEs typical of the pediatric population studied. Of the 69 subjects treated with rFVIIIFc, 59 (85.5%) reported at least one AE on study, giving a total of 213 AEs (Table 2). The majority of the AEs were judged by the investigator to be unrelated to rFVIIIFc treatment. Two non-serious AEs (myalgia and erythematous rash) were judged by investigators to be related to rFVIIIFc (Table S3). No subjects discontinued rFVIIIFc because of an AE; one subject withdrew prior to treatment with rFVIIIFc, owing to an event of *Klebsiella* sepsis in the

Table 1 Baseline characteristics of study subjects

Age cohort	< 6 years of age (N = 36)	6 years to < 12 years of age (N = 35)	Total (N = 71*)
Age (years), median (minimum, maximum)	4.0 (1, 5)	8.0 (6, 11)	5.0 (1, 11)
Weight (kg), median (minimum, maximum)	17.25 (13.0, 23.8)	31.35 (19.1, 59.6)	21.30 (13.0, 59.6)
Race, n (%)			
White	24 (66.7)	24 (68.6)	48 (67.6)
Black	4 (11.1)	5 (14.3)	9 (12.7)
Asian	4 (11.1)	1 (2.9)	5 (7.0)
Other	4 (11.1)	5 (14.3)	9 (12.7)
Geographic region, n (%)			
Europe	17 (47.2)	15 (42.9)	32 (45.1)
North America	9 (25.0)	11 (31.4)	20 (28.2)
Other	10 (27.8)	9 (25.7)	19 (26.8)
F8 genotype, n (%)			
Intron 22 inversion	17 (47.2)	9 (25.7)	26 (36.6)
Frameshift	9 (25.0)	7 (20.0)	16 (22.5)
Missense	5 (13.9)	7 (20.0)	12 (16.9)
Nonsense	2 (5.6)	7 (20.0)	9 (12.7)
Intron 1 inversion	1 (2.8)	2 (5.7)	3 (4.2)
Unknown	1 (2.8)	2 (5.7)	3 (4.2)
Not found	1 (2.8)	1 (2.9)	2 (2.8)
Prestudy FVIII regimen, n (%)			
Episodic	3 (8.3)	5 (14.3)	8 (11.3)
Prophylaxis	33 (91.7)	30 (85.7)	63 (88.7)
Once weekly	3 (9.1)	0 (0.0)	3 (4.8)
Twice weekly	7 (21.2)	6 (20.0)	13 (20.6)
Three times weekly	15 (45.5)	16 (53.3)	31 (49.2)
Every other day	8 (24.2)	8 (26.7)	16 (25.4)
Estimated total number of bleeds in the prior 12 months, median (minimum maximum)	2 (0, 16)	4 (0, 36)	2 (0, 36)
One or more target joints, n (%)	6 (16.7)	7 (20.0)	13 (18.3)
Family history of inhibitors, n (%)	3 (8.3)	3 (8.6)	6 (8.5)

*Sixty-nine of 71 subjects were exposed to recombinant factor VIII Fc fusion protein (rFVIIIFc) during the study; two subjects received a dose of prestudy factor VIII (FVIII) for pharmacokinetic evaluation, but withdrew prior to receiving rFVIIIFc.

setting of a central venous catheter infection. Five subjects (7.2%) experienced a total of seven serious AEs on study (two subjects experienced two events each); none was judged by investigators to be related to rFVIIIFc (Table 2). There were no reports of anaphylaxis or vascular thrombotic events with rFVIIIFc, and no deaths.

PK analyses

Of the 60 subjects dosed with rFVIIIFc in the PK subgroup, 54 (< 6 years of age, $n = 23$; 6 years to < 12 years of age, $n = 31$) had evaluable PK data from the one-stage clotting assay. The activity–time profiles in both age cohorts, adjusted for baseline and residual prestudy FVIII activity, were adequately characterized by non-compartmental analysis, with moderate intersubject variability (Fig. S3). The mean terminal half-lives of rFVIIIFc in subjects < 6 years of age and 6 years to < 12 years of age were 12.67 h (95% CI 11.23–14.11) and 14.88 h (95% CI 11.98–17.77), respectively (Table 3). An age-dependent effect on clearance was observed, with mean clearance being higher in the younger cohort (3.60 mL h^{-1} kg^{-1} [95% CI 3.13–4.07]) than in the older cohort (2.78 mL h^{-1} kg^{-1} [95% CI 2.44–3.13]). Incremental recovery of rFVIIIFc

was consistent between the age cohorts (mean of approximately 2 IU dL^{-1} per IU kg^{-1}) (Table 3), which is similar to what has been observed previously with FVIII products [17,18].

A comparison of FVIII and rFVIIIFc PK was performed for 46 subjects (< 6 years of age, $n = 19$; 6 years to < 12 years of age, $n = 27$) for whom data for both products were available. As subjects used various FVIII products for the prestudy PK assessment, a formal summary comparing rFVIIIFc with prestudy FVIII PK was not planned. However, *post hoc* summaries were prepared for two of the most common prestudy treatments, i.e. Advate and Helixate/Kogenate (Fig. 3A,B). Across all prestudy products, an intrasubject comparison of rFVIIIFc and FVIII half-lives yielded ratios ranging from 0.79 to 2.98 (Fig. 3C,D).

Efficacy

Overall, 46.4% of subjects reported no bleeding events on study. The median ABRs were 1.96 (IQR 0.00–3.96) overall, and 0.00 (IQR 0.00–4.01) over the last 3 months of the study (among subjects with ≥ 24 weeks on study) (Table 4). Subjects who had previously been receiving

FVIII prophylaxis showed a lowering of their median ABR with rFVIIIFc (< 6 years of age, n = 32, prestudy, 1.50, on study, 0.00; 6 years to < 12 years of age, n = 30, prestudy, 2.50, on study, 2.01). Across both age cohorts, the median ABR was zero for spontaneous and traumatic bleeding episodes, and for spontaneous joint bleeding episodes (Table 4). In subjects with one or more target joints

Table 2 Summary of adverse events (AEs) (≥ 3% in the total population)

Preferred term	< 6 years $N = 35^*$	6 years to < 12 years $N = 34^*$	Total $N = 69^*$
Subjects with at least one AE, n (%)	31 (88.6)	28 (82.4)	59 (85.5)
Cough	7 (20.0)	6 (17.6)	13 (18.8)
Upper respiratory tract infection	7 (20.0)	2 (5.9)	9 (13.0)
Fall	4 (11.4)	2 (5.9)	6 (8.7)
Nasopharyngitis	2 (5.7)	4 (11.8)	6 (8.7)
Upper abdominal pain	1 (2.9)	4 (11.8)	5 (7.2)
Head injury	3 (8.6)	2 (5.9)	5 (7.2)
Headache	1 (2.9)	4 (11.8)	5 (7.2)
Vomiting	3 (8.6)	2 (5.9)	5 (7.2)
Diarrhea	3 (8.6)	1 (2.9)	4 (5.8)
Ear infection	2 (5.7)	2 (5.9)	4 (5.8)
Fatigue	1 (2.9)	3 (8.8)	4 (5.8)
Pain in extremity	2 (5.7)	2 (5.9)	4 (5.8)
Pharyngitis	2 (5.7)	2 (5.9)	4 (5.8)
Seasonal allergy	2 (5.7)	2 (5.9)	4 (5.8)
Tonsillitis	2 (5.7)	2 (5.9)	4 (5.8)
Arthralgia	2 (5.7)	1 (2.9)	3 (4.3)
Joint swelling	2 (5.7)	1 (2.9)	3 (4.3)
Pyrexia	1 (2.9)	2 (5.9)	3 (4.3)
Rhinorrhea	2 (5.7)	1 (2.9)	3 (4.3)
Viral upper respiratory tract infection	3 (8.6)	0	3 (4.3)
Subjects with at least one SAE, n (%)†	4 (11.4)	1 (2.9)	5 (7.2)

SAE, serious adverse event. *Based on number of subjects receiving at least one dose of recombinant factor VIII Fc fusion protein (rFVIIIFc). †Five of 69 subjects treated with rFVIIIFc (7.2%) experienced a total of seven SAEs after at least one dose of rFVIIIFc (two subjects experienced two events each): head injury in two subjects, and the remaining SAEs (fall, *Bacillus* infection, *Escherichia* infection, croup infectious, and metapneumovirus infection) in one subject each.

at baseline, the median ABR for the 12 months prior to the study was 8.0 (IQR 4.0–11.0), as compared with 0.0 (IQR 0.0–5.8) on study (Table 4).

The median average dosing interval for rFVIIIFc prophylaxis was 3.5 days (Table 5). The majority (89.9%) of subjects made no changes to their dosing interval over the course of the study, indicating that the dosing regimens selected for study were appropriate for a pediatric population. As compared with their previous FVIII prophylactic regimen, 46 of 62 subjects (74.2%) reduced their dosing frequency with rFVIIIFc (Table S2). The median average weekly rFVIIIFc dose over the course of the study was 88.11 IU kg^{-1} weekly (IQR 80.29–103.08). Among subjects previously receiving FVIII prophylaxis, there was a trend towards a lower median average weekly dose with rFVIIIFc than with FVIII (< 6 years of age, from 97.75 IU kg^{-1} weekly [IQR 76.50–119.00] prestudy, to 90.55 IU kg^{-1} weekly [IQR 83.19–104.59] on study; 6 years to < 12 years of age, from 102.50 IU kg^{-1} weekly [IQR 78.00–129.00] prestudy, to 87.66 IU kg^{-1} weekly [IQR 79.71–104.05] on study), whereas bleeding rates remained similar to, or lower than, those observed with the prior FVIII regimen.

A total of 86 bleeding episodes occurred in 37 subjects on study. A single infusion was sufficient to resolve the majority (81.4%) of bleeding episodes; 93.0% were resolved with one or two infusions. Among the infusions evaluated by subjects for response, 89.4% were rated as producing an excellent or good response. Overall, the median dose per infusion for resolution of a bleed was 49.7 IU kg^{-1} per bleeding episode (Table 5). There were no major surgical procedures performed with rFVIIIFc on study. In seven minor surgical procedures in seven subjects (three subcutaneous intravenous access device placement procedures, three dental extractions, and one colonoscopy/endoscopy), the hemostatic response with rFVIIIFc was evaluated by the investigator as excellent (n = 5) or good (n = 2).

Discussion

The results of this phase 3 pediatric rFVIIIFc study confirm the findings of a similar study in adults and

Table 3 Recombinant factor VIII Fc fusion protein (rFVIIIFc) pharmacokinetics

Pharmacokinetic parameter, mean (95% CI):	< 6 years (N = 23)	6 years to < 12 years (N = 31)
IR (IU dL^{-1} per IU kg^{-1})	1.92 (1.80–2.04)	2.44 (2.07–2.80)
$t_{1/2}$ (h)	12.67 (11.23–14.11)	14.88 (11.98–17.77)
CL (mL h^{-1} kg^{-1})	3.60 (3.13–4.07)	2.78 (2.44–3.13)
DNAUC (IU × h dL^{-1} per IU kg^{-1})	30.04 (26.45–33.63)	41.87 (34.00–49.75)
MRT (h)	17.24 (15.40–19.07)	20.90 (17.06–24.74)
V_{ss} (mL kg^{-1})	58.58 (54.90–62.27)	52.13 (45.25–59.01)

CI, confidence interval; CL, clearance; DNAUC, dose normalized area under the concentration–time curve; IR, incremental recovery; MRT, mean residence time; $t_{1/2}$, half-life; V_{ss}, volume of distribution at steady state. The pharmacokinetic data reported are arithmetic mean (95% CI), derived from non-compartmental analysis of the one-stage clotting assay data; the pharmacokinetic parameters obtained from the one-stage assay were consistent with results from the two-stage chromogenic assay (data not shown).
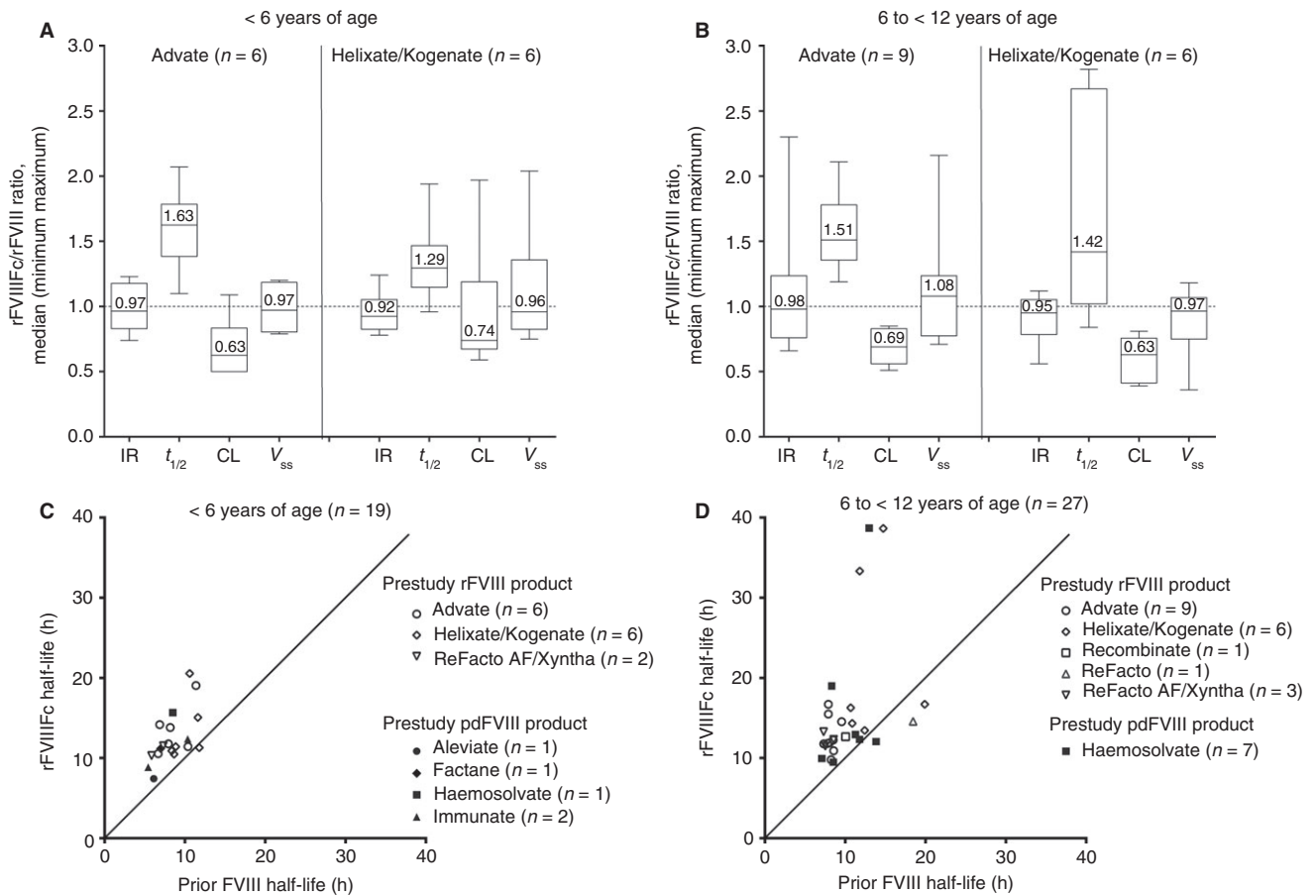
Fig. 3. Comparison of recombinant FVIII Fc fusion protein (rFVIIIFc) and prestudy recombinant FVIII pharmacokinetic (PK) parameters. A comparison of the FVIII PK parameters for rFVIIIFc versus prestudy FVIII was performed for 46 subjects (< 6 years of age, $n = 19$; 6 years to < 12 years of age, $n = 27$) with the one-stage clotting assay, and the ratio of rFVIIIFc PK to the PK of the prior FVIII for each subject was calculated. Representative summaries from subjects who received prestudy therapy with the most common prestudy treatments, Advate (A) or Helixate/Kogenate (B), are shown. The box represents the median and interquartile range, the 'whiskers' represent the minimum and maximum, and the reference unity line marks the point at which PK parameters for rFVIIIFc and prestudy FVIII are the same. For all subjects in the PK subgroup, an intrasubject comparison of FVIIIFc and prestudy FVIII PK was performed; data for the half-life comparison are shown by age cohort and prestudy FVIII product (C, D). CL, clearance; IR, incremental recovery; $t_{1/2}$, half-life; pdFVIII, plasma-derived FVIII; V_{ss}, volume of distribution at steady state. Data not shown for subjects for whom only historical FVIII PK data were available; these included one subject in the < 6-year cohort who used Advate prestudy, and two subjects in the 6-year to < 12-year cohort who used Refacto AF/Xyntha prestudy.

adolescents with severe hemophilia A, in which rFVIIIFc had a prolonged half-life relative to rFVIII [12,19]. Furthermore, rFVIIIFc was well tolerated and efficacious for the prophylaxis and treatment of bleeding in children < 12 years of age.

This is the first report of a pediatric study utilizing a long-acting factor therapy for routine prophylaxis. The benefits of early initiation of prophylaxis in children with hemophilia A include reduced bleeding, improved radiologic joint scores [3,4,20], an increased capacity for physical activity, and maintenance of bone health [21,22]. In this study, twice-weekly rFVIIIFc prophylaxis resulted in low ABRs for spontaneous bleeding episodes, including joint bleeding episodes, and 46.4% of study subjects reported no bleeding events on study. Subjects receiving a prestudy FVIII regimen of three intravenous infusions

per week experienced an approximately 33% reduction in annual infusions with twice-weekly rFVIIIFc prophylaxis. Additionally, rFVIIIFc was highly efficacious for the treatment of bleeding events, with 93.0% of subjects requiring only one to two rFVIIIFc infusions to manage a bleeding episode. These data are consistent with those expected for an efficacious FVIII therapy, and are in the range reported in prior rFVIII trials in pediatric populations [17,18].

Inhibitors develop in up to 30% of previously untreated patients with hemophilia A [23], and have also been reported in previously treated patients, although rarely [24,25]. No inhibitors developed with rFVIIIFc treatment, even among subjects with a genotype typically associated with inhibitor development. These results are similar to those reported in pediatric trials of previously

Table 4 Summary of annualized bleeding rate (ABR) by subgroup

ABR	Median	IQR	Range
Per subject, overall			
All subjects (n = 69)	1.96	0.00–3.96	0.0–27.2
< 6-year cohort (n = 35)	0.00	0.00–3.96	0.0–10.5
6-year to < 12-year cohort (n = 34)	2.01	0.00–4.04	0.0–27.2
Per subject, last 3 months on study*			
All subjects (n = 59)	0.00	0.00–4.01	0.0–12.0
< 6-year cohort (n = 26)	0.00	0.00–4.01	0.0–12.0
6-year to < 12-year cohort (n = 33)	0.00	0.00–4.01	0.0–8.0
By bleeding episode type and location			
Spontaneous			
All subjects (n = 69)	0.00	0.00–0.00	0.0–19.8
< 6-year cohort (n = 35)	0.00	0.00–0.00	0.0–7.9
6-year to < 12-year cohort (n = 34)	0.00	0.00–0.00	0.0–19.8
Spontaneous joint			
All subjects (n = 69)	0.00	0.00–0.00	0.0–14.8
< 6-year cohort (n = 35)	0.00	0.00–0.00	0.0–6.0
6-year to < 12-year cohort (n = 34)	0.00	0.00–0.00	0.0–14.8
Traumatic			
All subjects (n = 69)	0.00	0.00–2.04	0.0–7.9
< 6-year cohort (n = 35)	0.00	0.00–2.01	0.0–6.5
6-year to < 12-year cohort (n = 34)	0.00	0.00–2.12	0.0–7.9
By presence of target joints at baseline			
Subjects with ≥ 1 target joint (n = 13)			
Prestudy (12 months prior)	8.00	4.00–11.00	1.0–28.0
On study	0.00	0.00–5.83	0.0–27.2
Subjects with no target joints (n = 56)			
Prestudy (12 months prior)	2.00	1.00–4.00	0.0–36.0
On study	1.97	0.00–3.87	0.0–10.5

IQR, interquartile range. *Among 59 subjects (< 6-year cohort, n = 26; 6-year to < 12-year cohort, n = 33) with at least 24 weeks on study.

treated patients with other conventional or modified FVIII therapies [17,18,26]. Coupled with the lack of inhibitors observed in the A-LONG study (which included 110 subjects with ≥ 50 EDs)[12], these data suggest rFVIIIFc may be no more immunogenic than native FVIII molecules, with a low risk of inhibitor development in the previously treated patient population. Anti-rFVIIIFc binding antibodies were detected at baseline in some subjects in this study; however, these antibodies were not directed against the Fc portion of rFVIIIFc, and had no apparent clinical impact. These 'non-inhibitory' or 'non-neutralizing' anti-rFVIII antibodies have been previously reported in people with and without hemophilia [27–29], and their clinical significance remains unclear [30,31]. Further investigation is needed to fully understand the immunologic profile of rFVIIIFc, including the potential for inhibitor development with long-term treatment or in previously untreated patients, and whether rFVIIIFc has any immunologic advantages as compared with conventional therapies. The long-term safety and efficacy of rFVIIIFc are being studied in the ASPIRE extension study (ClinicalTrials.gov Identifier NCT01454739), and a study evaluating the safety and efficacy of rFVIIIFc in previously untreated patients is currently enrolling (ClinicalTrials.gov Identifier NCT02234323).

An age-dependent effect on rFVIIIFc PK was observed, as has been reported for rFVIII [9,32,33]. Despite the age-related increase in clearance, the half-life prolongation as compared with prestudy FVIII was consistent across age groups, and was consistent with the half-life extension previously reported in adults and adolescents [12,19]. There was greater variability in the prestudy/on-study half-life ratios in children than in adults, with some subjects showing a nearly three-fold prolongation of half-life, and others failing to show a beneficial effect (Fig. 3). Some of the observed variability can be explained by inherent physiologic differences in PK in pediatric patients [9,32]. Individual variability in the Fc-mediated recycling mechanism for rFVIIIFc is also possible, although this has not been investigated or reported for other Fc fusion proteins. Certain elements of the study design may also have contributed to the variability in prestudy/on-study half-life ratios, including the

Table 5 Recombinant factor VIII Fc fusion protein (rFVIIIFc) dosing summary

Age cohort	< 6 years (N = 35)	6 years to < 12 years (N = 34)	Total (N = 69)
rFVIIIFc prophylaxis			
Average weekly dose (IU kg^{-1}), median (IQR)			
Overall	91.63 (84.72–104.56)	86.88 (79.12–103.08)	88.11 (80.29–103.1)
Last 3 months*	101.86 (80.50–117.50)	88.97 (79.79–105.11)	92.51 (79.79–109.00)
Average dosing interval (days), median (IQR)			
Overall	3.50 (3.47–3.51)	3.49 (3.46–3.51)	3.49 (3.46–3.51)
Last 3 months*	3.49 (3.45–3.52)	3.49 (3.46–3.52)	3.49 (3.45–3.52)
Treatment of bleeding			
Dose per bleeding episode			
Bleeding episodes, n	38	48	86
Average dose per infusion(IU kg^{-1}), median (minimum, maximum)	51.35 (13.9, 91.3)	48.15 (14.0, 77.0)	49.69 (13.9, 91.3)
Total dose (IU kg^{-1}), median (minimum, maximum)	56.40 (13.9, 200.0)	53.49 (14.0, 196.6)	54.90 (13.9, 200.0)

IQR, interquartile range. *Among 59 subjects (<6 years cohort, n = 26; 6 to <12 years cohort, n = 33) with at least 24 weeks on study.

variety of FVIII products used for comparison, and variations in the availability of prestudy product information in certain geographic locations. Additionally, the study was designed to minimize blood draw volume in children, so a relatively sparse sampling schedule was used as compared with the adult study, which limited the information available for PK assessments [34]. It is known that von Willebrand factor (VWF) interacts with FVIII in the circulation, protecting it from proteolytic degradation and possibly providing structural stability to the protein [35]. However, this interaction also limits the ability to extend FVIII half-life beyond that of VWF (~ 10–25 h [36]) [19,37–39]. Whereas VWF levels have been shown to be correlated with rFVIIIFc half-life and extended dosing intervals for rFVIIIFc in adults [12,19,40], VWF levels were not measured in this study, in part because of the desire to limit blood sampling in pediatric subjects.

In addition to study design elements that may have contributed to variability in the PK data, this study had a few other limitations that are typical for pediatric hemophilia trials. The challenges of clinical trial design related to the recruitment of patients affected by hemophilia A [41] are magnified in a pediatric study. By design and as requested by regulatory agencies [15], the study population was limited to previously treated patients, and excluded subjects with a history of inhibitors, which further reduced the pool of subjects and yielded a population at lower risk for the development of inhibitors. Additionally, the study had a single arm, and thus did not concurrently compare rFVIIIFc with other FVIII products, and nor was it designed to maximize dosing intervals with rFVIIIFc. However, this trial enrolled a relatively large number of subjects representative of the global pediatric hemophilia A population, and, as the protocol allowed for dosing flexibility to represent a range of dosing regimens, the study was reflective of the real-world setting.

In conclusion, rFVIIIFc was well tolerated and efficacious in the prevention and treatment of bleeding events in children with severe hemophilia A, with no evidence of increased immunogenicity. The PK and efficacy results support the potential for extended-interval dosing and a reduced infusion frequency with rFVIIIFc as compared with conventional FVIII products, with excellent control of bleeding. The technological advance represented by Fc fusion may also have broader implications in other chronic diseases affecting pediatric populations where a reduction in injection frequency would be advantageous, and narrows the gap between current hemophilia therapies and new research focused on curative technologies that can lessen or eliminate the need for prophylaxis [42,43]. By mitigating the barriers presented by frequent infusions, rFVIIIFc may facilitate the more widespread adoption of early initiation of prophylaxis in younger subjects, which has the potential to positively impact on long-term outcomes.

Disclosure of Conflict of Interests

G. Young has received honoraria from Novo Nordisk and Biogen; and has consulted for Novo Nordisk, Biogen, Baxter, and Kedrion. J. Mahlangu has served on advisory boards for Amgen, Bayer, Genentech, and Novo Nordisk; and has received research funding from Biogen, Bayer, CSL Behring, and Novo Nordisk. R. Kulkarni has served on advisory boards for Biogen, Novo Nordisk, Baxter, Pfizer, Cangene, and Bayer; and has received research funding from Biogen, Novo Nordisk, and Octapharma. B. Nolan has received research funding from Biogen. R. Liesner has consulted for and/or received sponsorship from Bayer, Baxter, Novo Nordisk, Octapharma, and Pfizer; and has received research funding from Biogen, Octapharma, and Inspiration Biopharmaceuticals. J. Pasi has served on advisory boards for Bayer, BPL, Octapharma, Biogen, and Pfizer; and has received educational support and travel grants from Octapharma, Pfizer, Biogen, and Novo Nordisk. C. Barnes has served on advisory boards

Appendix
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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. rFVIIIFc dose and frequency modification scheme.
Fig. S2. Treatment of bleeding episodes on study.
Fig. S3. Mean FVIII activity over time following rFVIIIFc dosing.
Table S1. Inclusion and exclusion criteria (comprehensive list).
Table S2. Change in infusion frequency among subjects who had received prior FVIII prophylaxis ($N = 62$).
Table S3. Adverse events (AEs) judged by investigators to be related to rFVIIIFc treatment.

References

1 Manco-Johnson MJ, Riske B, Kasper CK. Advances in care of children with hemophilia. *Semin Thromb Hemost* 2003; **29**: 585–94.

2 Mannucci PM, Tuddenham EG. The hemophilias---from royal genes to gene therapy. *N Engl J Med* 2001; **344**: 1773–9.

3 Manco-Johnson MJ, Abshire TC, Shapiro AD, Riske B, Hacker MR, Kilcoyne R, Ingram JD, Manco-Johnson ML, Funk S, Jacobson L, Valentino LA, Hoots WK, Buchanan GR, DiMichele D, Recht M, Brown D, Leissinger C, Bleak S, Cohen A, Mathew P, *et al*. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med* 2007; **357**: 535–44.

4 Nilsson IM, Berntorp E, Lofqvist T, Pettersson H. Twenty-five years' experience of prophylactic treatment in severe haemophilia A and B. *J Intern Med* 1992; **232**: 25–32.

5 National Hemophilia Foundation. Medical and Scientific Advisory Council (MASAC) recommendation concerning prophylaxis (regular administration of clotting factor concentrate to prevent bleeding), 2007. http://www.hemophilia.org/NHFWeb/MainPgs/MainNHF.aspx?menuid=57{00AMP00}contentid=1007. Accessed 30 January 2015.

6 Hacker MR, Geraghty S, Manco-Johnson M. Barriers to compliance with prophylaxis therapy in haemophilia. *Haemophilia* 2001; **7**: 392–6.

7 Zappa S, McDaniel M, Marandola J, Allen G. Treatment trends for haemophilia A and haemophilia B in the United States: results from the 2010 practice patterns survey. *Haemophilia* 2012; **18**: e140–53.

8 Fogarty PF. Biological rationale for new drugs in the bleeding disorders pipeline. *Hematology Am Soc Hematol Educ Program* 2011; **2011**: 397–404.

9 Bjorkman S, Oh M, Spotts G, Schroth P, Fritsch S, Ewenstein BM, Casey K, Fischer K, Blanchette VS, Collins PW. Population pharmacokinetics of recombinant factor VIII: the relationships of pharmacokinetics to age and body weight. *Blood* 2012; **119**: 612–18.

10 Peters RT, Toby G, Lu Q, Liu T, Kulman JD, Low SC, Bitonti AJ, Pierce GF. Biochemical and functional characterization of a recombinant monomeric factor VIII Fc fusion protein. *J Thromb Haemost* 2013; **11**: 132–41.

11 Rath T, Baker K, Dumont JA, Peters RT, Jiang H, Qiao SW, Lencer WI, Pierce GF, Blumberg RS. Fc-fusion proteins and FcRn: structural insights for longer-lasting and more effective therapeutics. *Crit Rev Biotechnol* 2013 [Epub ahead of print].

12 Mahlangu J, Powell JS, Ragni MV, Chowdary P, Josephson NC, Pabinger I, Hanabusa H, Gupta N, Kulkarni R, Fogarty P, Perry D, Shapiro A, Pasi KJ, Apte S, Nestorov I, Jiang H, Li S, Neelakantan S, Cristiano LM, Goyal J, *et al*. Phase 3 study of recombinant factor VIII Fc fusion protein in severe hemophilia A. *Blood* 2013; **123**: 317–25.

13 Gouw SC, van den Berg HM, Oldenburg J, Astermark J, de Groot PG, Margaglione M, Thompson AR, van Heerde W, Boekhorst J, Miller CH, le Cessie S, van der Bom JG. F8 gene mutation type and inhibitor development in patients with severe hemophilia A: systematic review and meta-analysis. *Blood* 2012; **119**: 2922–34.

14 Margaglione M, Castaman G, Morfini M, Rocino A, Santagostino E, Tagariello G, Tagliaferri AR, Zanon E, Bicocchi MP, Castaldo G, Peyvandi F, Santacroce R, Torricelli F, Grandone E, Mannucci PM; the AICE-Genetics Study Group. The Italian AICE-Genetics hemophilia A database: results and correlation with clinical phenotype. *Haematologica* 2008; **93**: 722–8.

15 European Medicines Agency. Guideline on clinical investigation of recombinant and human plasma-derived factor VIII products.

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109692.pdf. Accessed 5 June 2014.

16 United States Food and Drug Administration. Guidance for industry: assay development for immunogenicity testing of therapeutic proteins. December 2009. http://www.fda.gov/downloads/Drugs/Guidances/UCM192750.pdf. Accessed 5 June 2014.

17 Baxter Healthcare Corporation. Advate [antihemophilic factor (recombinant), plasma/albumin-free method] (package insert). July 2012. http://www.advate.com/assets/pdf/advate_iri_pi.pdf. Accessed 17 July 2014.

18 Kulkarni R, Karim FA, Glamocanin S, Janic D, Vdovin V, Ozelo M, Rageliene L, Carboni E, Laguna P, Dobaczewski G, Seremetis S, Lindblom A, Santagostino E. Results from a large multinational clinical trial (guardian3) using prophylactic treatment with turoctocog alfa in paediatric patients with severe haemophilia A: safety, efficacy and pharmacokinetics. *Haemophilia* 2013; **19**: 698–705.

19 Powell JS, Josephson NC, Quon D, Ragni MV, Cheng G, Li E, Jiang H, Li L, Dumont JA, Goyal J, Zhang X, Sommer J, McCue J, Barbetti M, Luk A, Pierce GF. Safety and prolonged activity of recombinant factor VIII Fc fusion protein in hemophilia A patients. *Blood* 2012; **119**: 3031–7.

20 Gringeri A, Lundin B, von Mackensen S, Mantovani L, Mannucci PM; ESPRIT Study Group. A randomized clinical trial of prophylaxis in children with hemophilia A (the ESPRIT Study). *J Thromb Haemost* 2011; **9**: 700–10.

21 Khawaji M, Astermark J, Akesson K, Berntorp E. Physical activity for prevention of osteoporosis in patients with severe haemophilia on long-term prophylaxis. *Haemophilia* 2010; **16**: 495–501.

22 Ross C, Goldenberg NA, Hund D, Manco-Johnson MJ. Athletic participation in severe hemophilia: bleeding and joint outcomes in children on prophylaxis. *Pediatrics* 2009; **124**: 1267–72.

23 DiMichele D. Inhibitors: resolving diagnostic and therapeutic dilemmas. *Haemophilia* 2002; **8**: 280–7.

24 Gringeri A, Tagliaferri A, Tagariello G, Morfini M, Santagostino E, Mannucci P, ReFacto ASG. Efficacy and inhibitor development in previously treated patients with haemophilia A switched to a B domain-deleted recombinant factor VIII. *Br J Haematol* 2004; **126**: 398–404.

25 Xi M, Makris M, Marcucci M, Santagostino E, Mannucci PM, Iorio A. Inhibitor development in previously treated hemophilia A patients: a systematic review, meta-analysis, and meta-regression. *J Thromb Haemost* 2013; **11**: 1655–62.

26 Valentino LA, Negrier C, Kohla G, Tiede A, Liesner R, Hart D, Knaub S. The first recombinant FVIII produced in human cells---an update on its clinical development programme. *Haemophilia* 2014; **20**(Suppl. 1): 1–9.

27 Batlle J, Gomez E, Rendal E, Torea J, Loures E, Couselo M, Vila P, Sedano C, Tusell X, Magallon M, Quintana M, Gonzalez-Boullosa R, Lopez-Fernandez MF. Antibodies to factor VIII in plasma of patients with hemophilia A and normal subjects. *Ann Hematol* 1996; **72**: 321–6.

28 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**: 106–12.

29 Krudysz-Amblo J, Parhami-Seren B, Butenas S, Brummel-Ziedins KE, Gomperts ED, Rivard GE, Mann KG. Quantitation of anti-factor VIII antibodies in human plasma. *Blood* 2009; **113**: 2587–94.

30 Dazzi F, Tison T, Vianello F, Radossi P, Zerbinati P, Carraro P, Poletti A, Girolami A. High incidence of anti-FVIII antibodies against non-coagulant epitopes in haemophilia A patients: a possible role for the half-life of transfused FVIII. *Br J Haematol* 1996; **93**: 688–93.

31 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**: 385–92.

32 van Dijk K, van der Bom JG, Lenting PJ, de Groot PG, Mauser-Bunschoten EP, Roosendaal G, Grobbee DE, van den Berg HM. Factor VIII half-life and clinical phenotype of severe hemophilia A. *Haematologica* 2005; **90**: 494–8.

33 Bjorkman S, Folkesson A, Jonsson S. Pharmacokinetics and dose requirements of factor VIII over the age range 3–74 years: a population analysis based on 50 patients with long-term prophylactic treatment for haemophilia A. *Eur J Clin Pharmacol* 2009; **65**: 989–98.

34 Bjorkman S, Blanchette VS, Fischer K, Oh M, Spotts G, Schroth P, Fritsch S, Patrone L, Ewenstein BM, Advate Clinical Program G, Collins PW. Comparative pharmacokinetics of plasma- and albumin-free recombinant factor VIII in children and adults: the influence of blood sampling schedule on observed age-related differences and implications for dose tailoring. *J Thromb Haemost* 2010; **8**: 730–6.

35 Terraube V, O'Donnell JS, Jenkins PV. Factor VIII and von Willebrand factor interaction: biological, clinical and therapeutic importance. *Haemophilia* 2010; **16**: 3–13.

36 Gallinaro L, Cattini MG, Sztukowska M, Padrini R, Sartorello F, Pontara E, Bertomoro A, Daidone V, Pagnan A, Casonato A. A shorter von Willebrand factor survival in O blood group subjects explains how ABO determinants influence plasma von Willebrand factor. *Blood* 2008; **111**: 3540–5.

37 Kumar S, Liu T, Kulman JD, Chang P-Y, Ding S, Bardan S, Moore N, Patarroyo-White S, Pierce GF, Schellenberger V, Jiang H, Mei B, Peters RT, editors. Effect of unstructured polypeptide insertions on the recombinant expression of human factor VIII. Presented at: International Society on Thrombosis and Haemostasis—24th Congress; June 29–July 4, 2013; Amsterdam, The Netherlands. *J Thromb Haemost* 2013; **11**: 711.

38 Mei B, Pan C, Jiang H, Tjandra H, Strauss J, Chen Y, Liu T, Zhang X, Severs J, Newgren J, Chen J, Gu JM, Subramanyam B, Fournel MA, Pierce GF, Murphy JE. Rational design of a fully active, long-acting PEGylated factor VIII for hemophilia A treatment. *Blood* 2010; **116**: 270–9.

39 Tiede A, Brand B, Fischer R, Kavakli K, Lentz SR, Matsushita T, Rea C, Knobe K, Viuff D. Enhancing the pharmacokinetic properties of recombinant factor VIII: first-in-man trial of glycoPEGylated recombinant factor VIII in patients with hemophilia A. *J Thromb Haemost* 2013; **11**: 670–8.

40 Fogarty PF, Powell JS, Kruse-Jarres R, Mahlangu J, Perry D, McRae S, Potts J, Li S, Brennan A, Pierce GF, editors. Analysis of baseline characteristics and 5-day dosing interval with rFVIIIFc in the phase 3 A-LONG study. Presented at: Thrombosis and Hemostasis Summit of North America; April 10–12, 2014; Chicago, IL, USA. *Am J Hematol* 2014; **89**: E35.

41 DiMichele DM, Blanchette V, Berntorp E. Clinical trial design in haemophilia. *Haemophilia* 2012; **18**(Suppl. 4): 18–23.

42 McIntosh J, Lenting PJ, Rosales C, Lee D, Rabbanian S, Raj D, Patel N, Tuddenham EG, Christophe OD, McVey JH, Waddington S, Nienhuis AW, Gray JT, Fagone P, Mingozzi F, Zhou SZ, High KA, Cancio M, Ng CY, Zhou J, et al. Therapeutic levels of FVIII following a single peripheral vein administration of rAAV vector encoding a novel human factor VIII variant. *Blood* 2013; **121**: 3335–44.

43 Nathwani AC, Tuddenham EG, Rangarajan S, Rosales C, McIntosh J, Linch DC, Chowdary P, Riddell A, Pie AJ, Harrington C, O'Beirne J, Smith K, Pasi J, Glader B, Rustagi P, Ng CY, Kay MA, Zhou J, Spence Y, Morton CL, et al. Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. *N Engl J Med* 2011; **365**: 2357–65.