Supplemental Data

This Supplement has been provided by the authors to give readers additional information about their work.

Supplement to: J. Mahlangu, J. Powell, M. Ragni, et al.

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

Supplemental Data

Contents

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*Indicates investigators who screened patients but did not enroll any subjects into the study.

Author Contributions

Drs. Pierce, Luk, Jiang, and Ms. Nugent contributed to the design and conceptualization of the research, design of data analyses, interpretation of data, and drafting and revising the manuscript. Drs. Mahlangu, Apte, Chowdary, Fogarty, Gupta, Hanabusa, Josephson, Kulkarni, Pabinger, Pasi, Perry, Powell, Ragni, and Shapiro contributed to the data collection, design of data analyses, interpretation of data, and drafting and revising of the manuscript. Drs. Nestorov, Li, Cristiano, Neelakantan, Goyal, Sommer, Dumont, Brennan, and Vigliani contributed to the design of data analyses, data collection, interpretation of data, and drafting and revising of the manuscript. Mr. Dodd performed the statistical analyses and contributed to the interpretation of data and revision of the manuscript. All authors had access to the data and vouch for the completeness and accuracy of the data. The first draft of the manuscript was co-written by Drs. Brennan, Pierce, and Vigliani, with input from all coauthors, and editorial and medical writing assistance from a medical writer funded by the sponsor. All authors approved the final content of the manuscript and made the decision to submit it for publication, and take responsibility for the content and integrity of this article.

Additional Methodological Details

Full inclusion and exclusion criteria (comprehensive list)

Inclusion criteria	Exclusion criteria
• Provided written informed consent and any authorizations required by local law (eg, Protected Health Information). Parental or guardian consent was required for patients who were younger than 18 years; • Male and ≥12 years of age and weighed at least 40 kg; • Had severe hemophilia A defined as <1 IU/dL (<1%) endogenous FVIII activity as determined from the central laboratory at the time of screening. If the screening result was >1%, then the severity of hemophilia A was confirmed by documented historical evidence from a certified clinical laboratory demonstrating ≤1% factor VIII coagulant activity (FVIII:C) from the medical record or from a documented genotype known to produce severe hemophilia A; • Was a previously treated patient, defined as having at least 150 exposure days (EDs) to any recombinant or plasma-derived FVIII product (fresh frozen plasma treatment was not considered in the count of the documented EDs); • Had no measurable inhibitor activity in 2 consecutive samples and absence of clinical signs or symptoms of decreased response to FVIII administration; • Had bleeding events and/or treatment with FVIII during the prior 12 weeks, as documented in the patients' medical records; • Patient or a surrogate was willing and able to complete training in the use of the electronic patient diary and to use it throughout the study; • For patients entering arm 1: were on a prophylaxis regimen at least 2 times per week with an FVIII product or on an episodic regimen with ≥12 bleeding episodes in the 12 months prior to day 0 to ensure a severe phenotype; • For patients entering arms 2 or 3: were on an on-demand regimen with ≥12 bleeding episodes in the 12 months prior to day 0; • A platelet count ≥100 × 10^9 cells/L; • CD4 lymphocytes >200 mm^3 and viral load of <400 copies/mL, if known as HIV antibody-positive.	• Prior history of or current detectable inhibitor as defined by the reporting laboratory (family history of inhibitors did not exclude the patient). A positive inhibitor value was ≥0.6 Bethesda units (BU)/mL (≥1.0 BU/mL only for laboratories with a historical lower sensitivity cut-off for inhibitor detection of 1.0 BU/mL); • Other coagulation disorder(s) in addition to hemophilia A; • History of hypersensitivity or anaphylaxis associated with any FVIII or IV immunoglobulin administration; • For the pharmacokinetic comparison subgroup only: known hypersensitivity to mouse or hamster proteins; • Was taking (or likely to require during the study) acetylsalicylic acid or ibuprofen; • Concurrent systemic treatment with immunosuppressive drugs within 12 weeks prior to day 0; • Major surgery within the previous 8 weeks; • Unable to enter accurate and timely information regarding injections and bleeding episodes into an electronic patient diary and without adequate parental/caregiver support to manage this (per the investigator's judgment); • Unable or unwilling to refrain from taking additional prophylactic doses of FVIII prior to sports activities or an increase in physical activity; • Currently enrolled or enrolled within the previous 30 days in any other clinical trial involving investigational drugs; • Any concurrent clinically significant major disease or other unspecified reasons that, in the opinion of the investigator, made the patient unsuitable for participation in the study; • Abnormal renal function defined as serum creatinine >2.0 mg/dL; • Active hepatic disease defined as an aspartate aminotransferase or alanine aminotransferase greater than 5 times the upper limit of normal; • Serum bilirubin greater than 3 times the upper limit of normal.

Assignment of treatment

Following a 96-hour washout and subsequent screening period, subjects were assigned to treatment arms according to the standard of care and investigator decision, following discussion with each subject. Subjects receiving a prophylaxis regimen prior to study entry entered only into arm 1 (individualized prophylaxis). Subjects who were on an episodic regimen had the option to enter arm 1 or to be randomized into either arm 2 (weekly prophylaxis) or arm 3 (episodic regimen). Randomization into arm 2 or 3 was stratified based on the number of bleeding episodes reported by the subject during the 12 months prior to screening.

Subjects in any treatment arm could be enrolled in the surgery subgroup according to the following eligibility criteria: required major surgery; ≥12 EDs to rFVIIIFc with a negative inhibitor titer following this period and within 4 weeks prior to surgery; and completed, at minimum, pharmacokinetic sampling. Major surgery was defined as any surgical procedure (elective or emergent) that usually, but not always, involves general anesthesia and/or respiratory assistance in which a major body cavity is penetrated and exposed, or a substantial impairment of physical or physiologic functions is produced (eg, laparotomy, thoracotomy, craniotomy, joint replacement, or limb amputation).

Study visit schedule

Study visits occurred at screening (≤8 weeks), baseline, week 7, week 14, week 28, week 38, and week 52. Additionally, subjects had a 30-day follow-up telephone call unless they enrolled in the ongoing extension study ASPIRE (ClinicalTrials.gov number, NCT01454739). In the surgery subgroup, subjects had assessments 4 weeks prior to surgery, the day of surgery, and 24 hours postoperatively.

Sequential pharmacokinetics subgroup

Length of sampling times for rFVIII and rFVIIIFc were based upon previously reported half-lives,[1, 2] allowing sufficient time for decay (normally 3–5 times the previously observed half-life) for accurate description of pharmacokinetics. Following a 96-hour washout period, subjects in the sequential pharmacokinetics subgroup in arm 1 received an injection of 50 IU/kg rFVIII (Advate®) and underwent sampling up to 72 hours as follows: preinjection, 30 (±3) minutes, 1 hour (±15 minutes), 6 (±1) hours, 24 (±2) hours (day 1), 48 (±2) hours, and 72 (±2) hours (day 3) from the start of the injection.

After the rFVIII dose, subjects underwent a ≥96-hour washout. Subjects then received a dose of 50 IU/kg of rFVIIIFc and underwent sampling for rFVIIIFc pharmacokinetic profiling as follows: preinjection and 30 (±3) minutes, 1 hour (±15 minutes), 6 (±1) hours, 24 (±2) hours (day 1), 72 (±2) hours (day 3), 96 (±2) hours (day 4), and 120 (±2) hours (day 5) from the start of the injection. The rFVIIIFc pharmacokinetic assessment was repeated 12 to 24 weeks later, following the same sampling schedule.

Each subject had to have completed pharmacokinetic sampling through at least the 48-hour time point for rFVIII and the 72-hour time point for rFVIIIFc to be included in the pharmacokinetic analysis set.

The sequential pharmacokinetics subgroup consisted of all subjects who had evaluable pharmacokinetic profiles for both rFVIII and baseline rFVIIIFc and/or evaluable pharmacokinetic profiles for both baseline rFVIIIFc and the repeat rFVIIIFc profile.

Prophylaxis dose and interval titration, and episodic treatment of bleeding episodes

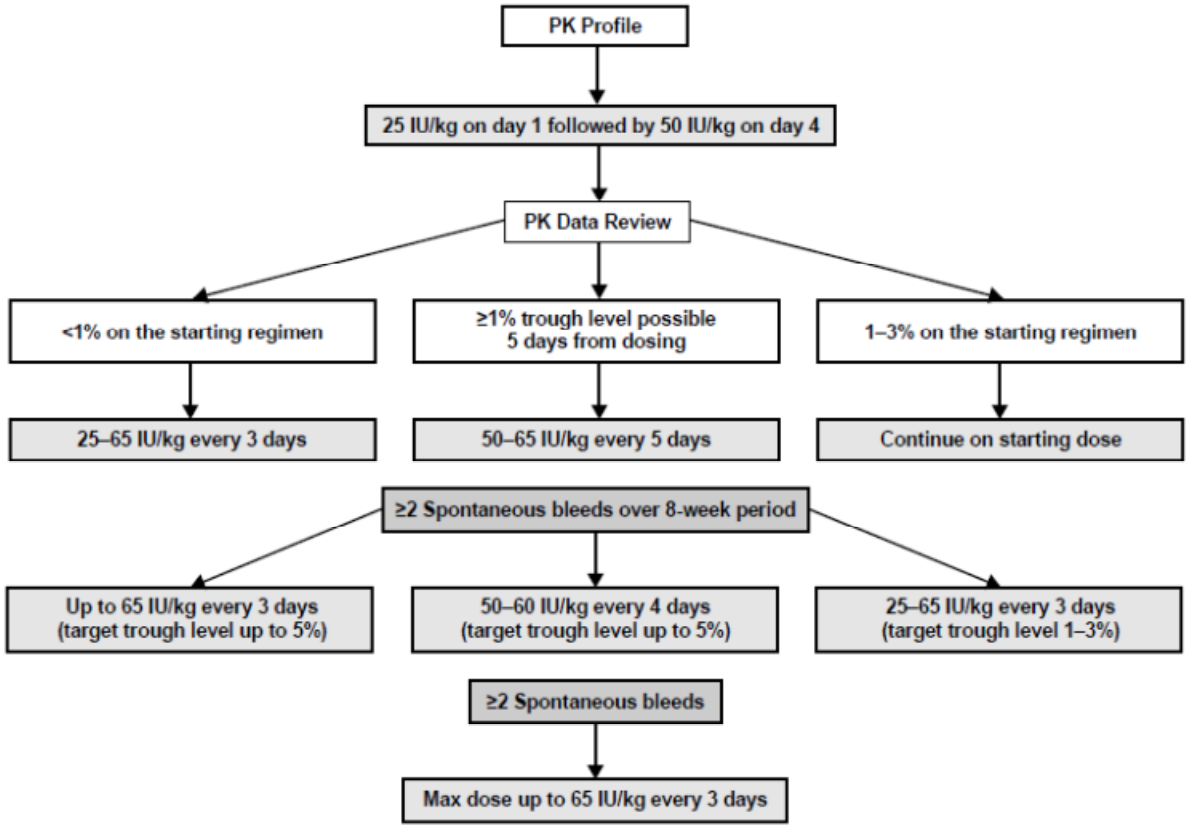
Arm 1 (individualized prophylaxis): subjects initially received twice-weekly dosing (equivalent to every 3.5 days) of 25 IU/kg on day 1 and 50 IU/kg on day 4. Pharmacokinetic data were collected before the second dose and were reviewed by the study committee (generally within 4 weeks of data availability), to allow for subsequent adjustments to dose and dosing interval as described in the Methods.

Arm 2 (weekly prophylaxis): subjects received a fixed dose of 65 IU/kg rFVIIIFc every 7 days with no dose or interval adjustment.

Arm 3 (episodic regimen): subjects received rFVIIIFc doses between 10 and 50 IU/kg, according to bleed severity using published guidelines.[3]

In arm 3 (episodic treatment) and in any subjects who experienced bleeding episodes in arms 1 and 2, minor bleeding episodes were treated with 10–20 IU/kg, moderate to major bleeding episodes with 15–30 IU/kg, and major to life-threatening bleeding episodes with 40–50 IU/kg to target trough FVIII levels of 20%–40%, 30%–60%, and 80%–100%, respectively, based on the subject's pharmacokinetic profile.

Figure S1. **Dose modification in arm 1 (individualized prophylaxis).**



Efficacy period

In arms 1 and 2, the efficacy period started with the date and time of the first prophylactic dosage following the completed pharmacokinetic sampling period and ended with last dose administered (for prophylaxis or a bleeding episode) as recorded in the electronic case report forms or patient diaries. For arms 1 and 2, the interval of time in between the last dose before the repeated pharmacokinetics (arm 1 sequential pharmacokinetics subgroup) or surgical/rehabilitation period and the start of the pharmacokinetics or surgical/rehabilitation period were not attributed to any of the efficacy or pharmacokinetic periods.

In arm 3, the efficacy period started 1 minute following the last pharmacokinetic sampling time points and ended with either the date of last contact or the date of the last entry into the electronic patient diaries, whichever was later.

Definition of a target joint

A target joint was defined as a major joint (eg, hip, elbow, wrist, shoulder, knee, and ankle) into which repeated bleeding occurs (frequency of ≥3 bleeding episodes into the same joint in a consecutive 6-month period).

Definition of bleeding episode

A standardized definition of a bleeding episode was used in this study. A bleeding episode started from the first sign of bleeding and ended 72 hours after the last treatment for the bleeding, within which any symptoms of bleeding at the same location or injections less than or equal to 72 hours apart, were considered the same bleeding episode. Any injection to treat the bleeding episode, taken more than 72 hours after the preceding one, was considered the first injection to treat a new bleeding episode at the same location. Any bleeding at a different location was considered a separate bleeding episode regardless of time from last injection. This definition has been proposed by the Subcommittee on Standards and Criteria, FVIII/FIX subcommittee of the International Society of Thrombosis and Hemostasis, and has been used by the PedNet multicenter study in hemophilia.[4, 5]

Annualized bleeding rates (ABRs)

The number of bleeding episodes was annualized for each subject using the following formula:

$$\text{ABR} = \frac{\text{Number of bleeding episodes during efficacy period}}{\text{Total number of days during the efficacy period}} \times 365.25$$

All subjects who received at least 1 efficacy dose of rFVIIIFc were included.

Assessment of ABRs, the primary efficacy outcome, was to be performed using a Poisson regression model with treatment arm as covariate and no overdispersion (otherwise, a negative binomial model, which accounts for overdispersion, was to be used). At the target sample size (~144 subjects enrolled, based on inhibitor incidence criteria [see below]), the models were projected to have greater than 90% power at the 2-sided .05 level of significance to detect a 60% reduction in annualized bleeding episodes between arm 1 and arm 3. This was considered based on the following:

- Minimum follow-up time for subjects in arm 1 is 26 weeks starting from the first prophylaxis dose (10 days after the first rFVIIIFc dose on study); minimum follow-up time for subjects in arm 3 is 26 weeks starting from day 0;
- Total follow-up time of each treatment arm was 1320 patient-weeks (104 subjects) for arm 1 and 400 patient-weeks (20 subjects) for arm 3;
- ABR for the population of subjects using episodic treatment is ≥10 bleeding episodes per subject per year; a ≥50% reduction in ABR was considered clinically important.

Treatment compliance

Compliance with treatment dosing was monitored and documented by site staff. For between-visit administration, subjects self-administered rFVIIIFc and documented treatment in the hand-held electronic diary, which was reviewed during periodic calls to the subject and at each visit by study site staff and the clinical monitor.

For subjects in arms 1 and 2, compliance with the prophylactic regimen was calculated in 2 ways, as dose compliance and as dosing interval compliance. Analyses of compliance included percentage of nominal doses taken per subject within the 80%–125% range, and the percentage of doses taken per subject within ±24 hours of the prescribed day. Subjects were considered compliant if the calculated compliance rate was at least 80%.

Analytical methods

FVIII activity in citrated plasma samples for rFVIII and rFVIIIFc was measured by the 1-stage clotting (activated partial thromboplastin time [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. The lower limit of quantification (LLOQ) for the 1-stage assay was 0.5 IU/dL. The accuracy was between 92% and 116% for the 1-stage assay, whereas the interassay coefficients of variation were <10%.

In addition to the aPTT assay, FVIII activity was measured by a chromogenic substrate assay using a commercially available kit (Aniara BIOPHEN FVIII:C) that complies with European Pharmacopoeia recommendations and was qualified for quantification of rFVIII and rFVIIIFc in human plasma. The chromogenic assay was calibrated against normal human reference plasma (Instrumentation Laboratories Catalogue no. ORKE45), which had a potency assigned against the WHO 6th IS for human plasma. The chromogenic assay had the LLOQ of 0.8 IU/dL and the interassay coefficients of variation were <15%.

Pharmacokinetics analyses

Baseline and residual drug corrections were performed on the observed FVIII activity results from both assays. The resulting corrected FVIII activity over time profiles were analyzed using a mixed 1- and 2-compartmental model in Phoenix WinNonLin (version 6.2.1.51). The optimal model for each subject was selected based on Akaike information criterion[6] and the precision of the parameter estimates. Assuming the standard deviation of differences was ≤0.45 for primary pharmacokinetic endpoints, comparison of log-transformed endpoints using an analysis of variance model with factors for study treatment and subject would have ≥90% power to detect a 1.5-fold increase in rFVIIIFc over rFVIII.

Detection of inhibitors (neutralizing antibodies)

The Nijmegen-modified Bethesda assay to detect neutralizing antibodies was performed at screening, baseline, and each visit during study treatment to monitor for the development of an inhibitor. Following the first dose with rFVIIIFc, inhibitor testing in arms 1 and 2 was conducted at trough levels at each scheduled clinic visit, with trough defined as a point after the longest interval between scheduled doses. In addition, subjects in arms 1 and 2 were required to have inhibitor testing after 10 to 15 EDs to rFVIIIFc and again after they completed 50 to 75 EDs to rFVIIIFc. If these time points did not coincide with scheduled visits, additional visits were scheduled for the testing. Subjects in arm 2 may not have achieved 50 EDs on this study. For arm 3, inhibitor testing was performed at each scheduled visit, at least 48 hours after the previous injection. Formation of an inhibitor was defined as a neutralizing antibody value ≥0.6 BU/mL, identified and confirmed by retesting of a second sample within 2 to 4 weeks.

Analysis of inhibitor incidence

Considerations of acceptable inhibitor risk in clinical trials of previously treated FVIII patients[7] are made under the assumption that the occurrence of inhibitors in a clinical trial can be adequately modelled using the binomial distribution, which results in a 2-sided 95% confidence interval (CI) for the true inhibitor incidence of (1.9%–6.8%) using the exact (Clopper-Pearson) method.[8] That is, this criterion is equivalent to demonstrating at the 2-sided .05 level of significance that if 2 or fewer subjects experienced an inhibitor during the study (ie, the observed incidence is ≤1.9% when using only the 104 subjects with at least 50 EDs in the denominator), then the upper bound of an exact (Clopper-Pearson) 2-sided 95% CI would exclude ≥6.8%. Assuming that the true inhibitor incidence for the population eligible for this study is no greater than 1.0%, then there is at least 80% probability of no more than 2 subjects out of 144 developing an inhibitor.

Detection of non-neutralizing antibodies (rFVIIIFc binding antibodies)

Monitoring for non-neutralizing antibodies (NNAs) that bind to rFVIIIFc was performed at the same time points as testing for inhibitors. A bridging assay format was employed to detect all possible classes of antibodies, with electrochemiluminescent readout on a Meso Scale Discovery® (MSD) instrument. Samples were positive if the signal was above a statistically derived cut point and confirmed by inhibition with excess rFVIIIFc product in at least 2 of 3 replicate assays. Positive samples were further characterized for binding specificity to rFVIII or Fc. This assay was ~20-fold more sensitive than the Nijmegen-modified Bethesda assay.

Five subjects were NNA-positive at screening; in all 5 cases, NNA titers declined over the course of the study, to the point that in 2 subjects the antibody was not detected at the final visit. Six subjects exhibited a negative NNA response at screening, followed by positive results after the first dose of rFVIIIFc. A negative confirmatory result was obtained in 4 of the 6 subjects who tested positive on study; 2 subjects remained NNA-positive at the last study visit. There was no evidence of a lack of efficacy in either the subjects with a positive result at screening or in the subjects with NNA-positive results on study.

Key laboratory tests

To ensure consistency of laboratory analyses, 1 central laboratory was used for each type of assay. Central sample management and analyses of clinical safety samples were performed at LabCorp (Cranford, NJ, USA). The 1-stage clotting assay and the Nijmegen-modified Bethesda assay were performed at Esoterix Laboratory Services Inc. (LabCorp, Englewood, CO, USA). FVIII chromogenic activity and NNA assays were conducted at the Assay Services Laboratory, Biogen Idec (Cambridge, MA, USA). Genotyping of samples was done at Hemostasis Lab, Puget Sound Blood Center (Seattle, WA, USA).

Study stopping rules

The study could conclude when all the following predefined criteria were met:

1) Thirteen subjects in the sequential pharmacokinetics subgroup in arm 1 had completed rFVIII pharmacokinetic profile, the rFVIIIFc day 0 pharmacokinetic profile, and a repeat 5-day rFVIIIFc pharmacokinetic profile 12 to 24 weeks later with adequate estimate of the terminal half-life.
2) A minimum of 104 subjects from any arm had completed 50 rFVIIIFc EDs with an inhibitor test result from the central laboratory following at least 50 EDs, and at least 50 of these subjects had an inhibitor test result from the central laboratory following 10 to 15 and 50 to 75 EDs.
3) ~20 subjects from arm 2 and 20 subjects from arm 3 had completed at least 28 (±2) weeks on study.
4) A minimum of 10 major surgeries had been conducted in at least 5 subjects.

When all of these criteria had been met, all ongoing subjects were asked to return to the clinic for end-of-study assessments.

Additional results

Table S1. Pharmacokinetic parameters of rFVIIIFc compared with rFVIII: two-compartmental model, one-stage clotting assay for subjects in the sequential pharmacokinetics subgroup (N = 28)

Pharmacokinetic parameter	rFVIIIFc Geometric mean (95% CI)	rFVIII Geometric mean (95% CI)	Intrasubject ratio Geometric mean (95% CI)	*P*
AUC normalized to dose, IU*h/dL per IU/kg	51.2 (45.0–58.4)	32.9 (29.3–36.9)	1.6 (1.5–1.7)	<.001
Elimination $t_{½}$, h	19.0 (17.0–21.1)	12.4 (11.1–13.9)	1.5 (1.4–1.7)	<.001
Clearance, mL/h/kg	2.0 (1.7–2.2)	3.0 (2.7–3.4)	0.6 (0.6–0.7)	<.001
Mean residence time, h	25.2 (22.7–27.9)	16.8 (15.2–18.6)	1.5 (1.4–1.6)	<.001
V_{ss}, mL/kg	49.1 (46.6–51.7)	51.2 (47.2–55.5)	1.0 (0.9–1.0)	.197
Incremental recovery, IU/dL per IU/kg	2.2 (2.1–2.4)	2.4 (2.2–2.5)	1.0 (0.9–1.0)	.025
Time to 1 IU/dL, d[*]	4.9 (4.4–5.5)	3.3 (3.0–3.7)	1.5 (1.4–1.6)	<.001
Time to 3 IU/dL, d)[†]	3.7 (3.3–4.1)	2.5 (2.2–2.7)	1.5 (1.4–1.6)	<.001

AUC indicates area under the curve; $t_{½}$, half-life; and V_{ss}, volume of distribution at steady state.
[*]Following injection of 50 IU/kg.

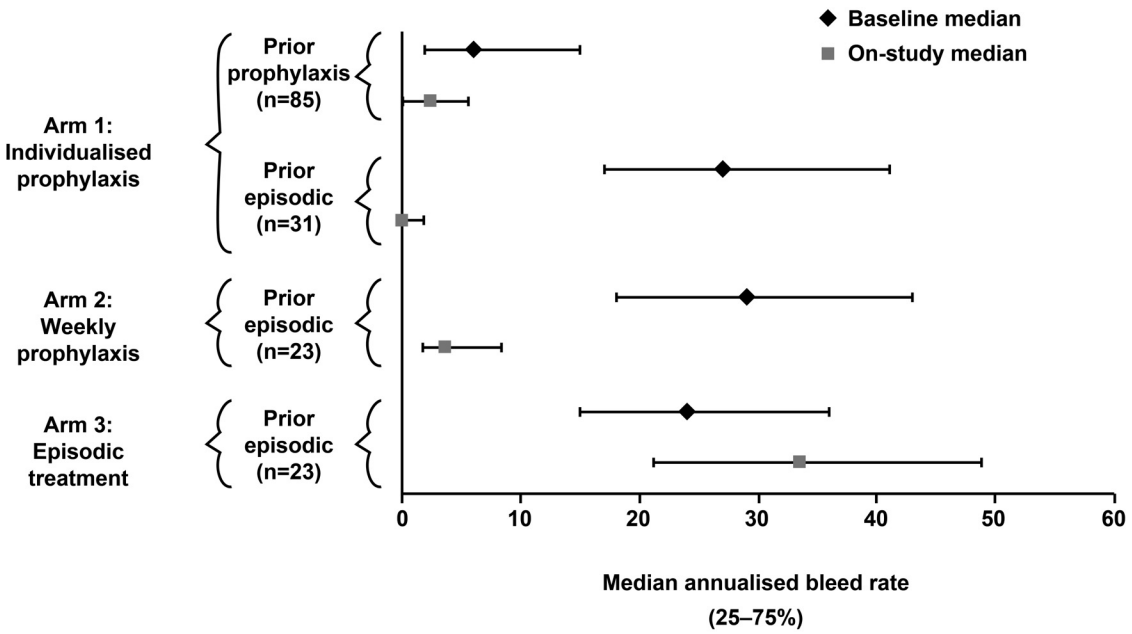
Table S2. Summary of efficacy in control of bleeding episodes

Total no. of new bleeding episodes	757
Injections to treat bleeding episodes, n (%)	
1 injection	661 (87.3)
2 injections	79 (10.4)
3 injections	13 (1.7)
Median dose per injection to treat a bleeding episode, IU/kg (IQR)	27.35 (22.73, 32.71)

IQR indicates interquartile range.

Figure S2. Number of bleeding episodes in the prior 12 months compared with the on-study ABR by prior FVIII regimen.

Compared with the estimated number of bleeding episodes in the 12 months prior to the study, the observed ABR for subjects in arm 1 was lower for both those on prior prophylaxis and those on prior episodic treatment. Likewise, subjects in arm 2 who were previously treated episodically exhibited reduced on-study ABRs compared with prestudy bleeding episodes. There was no significant difference in the number of prestudy versus on-study bleeding episodes for subjects in arm 3.


Baseline median
On-study median
Arm 1: Individualised prophylaxis
Prior prophylaxis (n=85)
Prior episodic (n=31)
Arm 2: Weekly prophylaxis
Prior episodic (n=23)
Arm 3: Episodic treatment
Prior episodic (n=23)
0
10
20
30
40
50
60
Median annualised bleed rate
(25–75%)

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