REVIEW

Therapeutic and routine prophylactic properties of rFactor VIII Fc (efraloctocog alfa, Eloctate®) in hemophilia A

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Abstract: rFVIIIFc (efraloctocog alfa, Eloctate®) is an extended half-life (EHL) factor VIII licensed for use in patients with hemophilia A for prophylaxis and treatment of bleeding and surgical episodes. Pharmacokinetic studies in adults have shown a mean 1.5-fold increase in half-life compared to full-length factor VIII. When compared to adults, the half-life is decreased by 8% in adolescents between 12 and 17 years, by 18% in children 6 to <12 years, and by 33% in children between the ages of 2 and <6 years. There is a considerable interindividual variation in the prolongation of the half-life particularly in children and across the age groups, the range extending from no increase to a 2.5-fold increase. In addition to age, von willebrand factor (VWF) antigen level has demonstrated a significant impact on rFVIIIFc half-life, with higher VWF levels associated with greater prolongation of half-life. The pivotal and pediatric clinical trials have demonstrated the efficacy and safety of rFVIIIFc for use in regular prophylaxis and in management of bleeds and surgery. In these studies, just under half the participants showed a zero annualized bleed rate (ABR), and the median ABR (1.6 in the pivotal study for the individualized prophylaxis arm) showed a further decrease in the extension study. On average, the patients required fewer infusions (reduced by at least a third), and the mean weekly consumption seems to be in keeping with standard recombinant factor VIII. EHL rFVIIIFc has made decreased infusion frequency a possibility. However, the interindividual variability in dose and infusion frequency highlights the need for a personalized approach based on individual patient's half-life and/or response to treatment.

Keywords: hemophilia A, rFVIIIFc, prophylaxis, FVIII, extended half-life factors, efraloctocog alfa

Background

Hemophilia A is an X-linked inherited bleeding disorder due to deficiency or absence of coagulation factor VIII, with an incidence of one in 5,000 male births and higher prevalence in high-income countries.[1] The plasma levels of factor VIII determine the clinical picture, and in patients with severe hemophilia the factor levels are <1%, and bleeding is characteristically spontaneous and recurrent and all tissues and organs are susceptible.[2,3] Before the introduction of replacement therapy, bleeding was the predominant cause of death and most patients with severe hemophilia did not survive beyond their second decade.[4] While replacement therapy has resulted in decreased mortality from bleeding, recurrent bleeding into joints and muscles continues to be seen, and joint arthropathy, muscle damage, and disability are the most frequent sequelae of the condition. The introduction of prophylaxis, characterized by self-infusion of factor VIII 2–4 times a week, has brought about a paradigm shift in the management of severe hemophilia. Most patients in developed countries can now hope to have near

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normal life expectancy with a variable disability, although there continues to be slight excess mortality in patients with severe hemophilia A.[5] A major aim of prophylaxis in the management of hemophilia is the prevention of joint damage and the arrest or slowing of progression of existing joint damage.

Despite tremendous advances in the management of hemophilia, there are several challenges that require the hemophilia community's attention.[6,7] Important challenges include access to treatment and the patient's burden of illness. The burden of illness is in part related to the time commitment required for regular prophylaxis and has a considerable influence on patient adherence and, at times, the nature of prophylaxis restricts full participation in society.[8] The frequency of infusion is often cited as an important factor that influences adherence and burden of illness, which in turn influences physician-prescribing behavior.[9] Numerous technologies have evolved in the last decade that have facilitated the development of factor VIII molecules with extended half-life (EHL) principally through decreased clearance. Decreased clearance is achieved through a combination of reduced proteolysis in peripheral blood, decreased renal and hepatic elimination, and decreased receptor-mediated endocytosis.[10] rFVIIIFc or efraloctocog alfa or Eloctate® (Biogen, Cambridge, MA, USA) is a recombinant factor VIII with an EHL due to decreased clearance mediated by fusion to an Fc dimer. It is licensed in both the USA and Europe for clinical use in patients with hemophilia A. This paper reviews the evolution of prophylaxis, current challenges in this area, published data from rFVIIIFc clinical trials to date, and potential clinical application.

The evolution of prophylaxis

World Federation of Hemophilia guidelines published in 2012 define prophylaxis as follows: "[...] the treatment by intravenous injection of factor concentrate to prevent anticipated bleeding".[11] The goal of prophylaxis is to increase FVIII to sufficient levels to prevent spontaneous bleeding events and resultant joint damage.[12] Both retrospective and prospective studies have provided evidence for the efficacy of prophylaxis and that it concomitantly improves the quality of life in affected patients.[13–17]

The concept of prophylaxis was first pioneered by a Swedish group in 1958[18] when they recognized the threshold effect of a FVIII level of 1% or above on bleeding phenotype, ie, the difference between a patient with severe and moderate hemophilia. Patients with moderate hemophilia, although still at risk of trauma-induced bleeds, did not experience

spontaneous bleeding. The Malmo prophylaxis regimen was introduced in the 1950s and accordingly children <2 years of age were treated with infusions of factor VIII on alternate days at a dose of 25–40 IU/kg, with the aim of changing their phenotype to that seen in moderate hemophilia patients.[18] The Malmo prophylaxis experience published in the early 1990s based on data from 60 patients demonstrated that the annualized bleed rate (ABR) was almost zero and that there was no orthopedic or radiological evidence of joint damage in patients in whom prophylaxis was initiated at a sufficiently young age. In those in whom joint damage was already present, deterioration could not be halted but the rate of progression could be slowed. Prophylaxis was introduced in the Netherlands a decade later in the late 1960s, but with a different approach. Mindful of the health economic cost implications of full-dose prophylaxis, lower doses at longer intervals were used and treatment tailored to the clinical picture and modified accordingly to prevent spontaneous joint bleeds. Follow-up comparative studies have demonstrated the superiority of high-dose prophylaxis regarding ABR and joint health but significantly show similar scores regarding the quality of life and social participation.[19] In both Sweden and the Netherlands, the doses used have increased since the inception of prophylaxis, but the full- and intermediate-dose regimens remain and illustrate that dose modification on an individual basis can still result in a satisfactory outcome. In addition, the approach of secondary prophylaxis has been successfully implemented with prophylaxis not initiated by age, but on clinical phenotype, ie, after the first joint bleed.[19–21]

In many other parts of the world, the practice of routine prophylaxis was slow to be implemented. This partly reflected issues of upfront cost and concerns about adherence and transfusion transmitted infections as well as the need for adequate venous access. However, it was increasingly recognized that episodic therapy could still result in the same annual factor concentrate consumption with patients disabled by hemophilic arthropathy.[22] Previous studies had compared results of prophylaxis against historical controls, but in 2007 the results of an American multicenter, randomized, open-label trial were published in which boys were enrolled from 1996 onwards.[23] Boys were randomly assigned either prophylaxis (25 IU/kg on alternate days) or an enhanced on-demand schedule comprising of at least three doses, totaling a minimum of 80 IU/kg for the treatment of a joint bleed. The primary outcome was evidence of joint damage (ankles, elbows, and knees) on radiological examination. At the age of 6 years (having been recruited below the age

of 30 months), 93% (versus 55%) of those on prophylaxis were deemed to have normal joints on imaging. The relative risk of joint damage seen on MRI was 6.1 (95% confidence interval [CI] 1.5–24.4) in those treated episodically, and the mean ABR was significantly higher in this group. Since the publication of this trial there is increasing uptake of prophylaxis, but this is by no means universal.

Current challenges relating to prophylaxis

Multiple studies have unequivocally demonstrated the superiority of prophylaxis as compared to on-demand or episodic therapy,[24] and this is now the gold standard. However, debate continues among clinicians over when prophylaxis should start, what dose should be used, and how frequently. It is increasingly recognized that a uniform approach can lead to undertreatment of some individuals and overtreatment of others. Questions arise as to the most appropriate factor VIII trough level to be aimed for as well as the impact of time spent below this trough. A hemophilia bleed-rate-probability model in patients receiving prophylaxis was described by Collins et al,[13] in which they predicted the probability of bleeding in a year in relation to the time spent at factor VIII levels of <1% in a week. The evidence showed that increasing time spent at <1% correlated with an increased bleed rate and hemarthrosis. Importantly, the bleed prediction in the probability model showed a relatively flat curve when the total duration of time spent at <1% per week was below 24 hours. This finding is corroborated by data from a cohort study that demonstrated that patients with moderate hemophilia develop bleeds at low frequency.[25] Factors that have a noticeable impact on the time spent at <1% are adherence, dose, and frequency of prophylaxis and an individual's biological half-life ($t_{1/2}$).

Most current prophylactic regimens are based on body weight, and efficacy and adequacy are monitored by trough levels in addition to the frequency of self-reported bleeds. A major challenge is transition of prophylactic regimens from fixed weight-based regimens to outcome-based personalized regimens. Outcomes can be patient reported or surrogate markers or a combination of both. In addition to factors that influence time spent at <1%, age and activity levels also influence bleeding phenotype. Patient adherence to the prescribed treatment and activity recommendations has a significant influence on the outcomes, and it has been suggested that decreasing the frequency of infusions will foster adherence and eventually improve the outcomes.[6] EHL factor VIII molecules offer such a potential and present the clinician and patient with opportunities that allow for personalized prophylactic regimens with patient-specific trough levels and infusion frequency that takes into account age, half-life, and patient lifestyle and acceptability.

Extending the half-life – the rFVIIIFc molecule

Biotechnology is currently going through the second wave of protein therapeutics, characterized by the manufacture of novel molecules with increased or prolonged activity and/or decreased immunogenicity, achieved by different methods.[10] The protein structure can be modified through targeted mutations, altered glycosylation, or by covalently attached moieties, eg, polyethylene glycol or polysialic acid, albumin, or immunoglobulin. Other approaches are changes to the formulation as seen with liposomes, polymeric microspheres/nanoparticles, and polylactic-co-glycolic acid microspheres. Advances in biomolecular and protein engineering have resulted in two broad strategies for extending the half-life of factor VIII and these include Fc fusion and PEGylation, and both strategies principally decrease the clearance of factor VIII. In Fc fusion, the extension of half-life is achieved through fusion with an immunoglobulin molecule with a known long half-life,[26] while in PEGylation, the physiochemical properties of the protein are changed through conjugation with hydrophilic polymers.[27–30]

Immunoglobulins and albumin constitute more than 90% of serum proteins, and their plasma concentration is a function of the rate of synthesis, the rate of degradation, and recycling pathways. A recycling pathway that is key to the plasma concentration of the above two proteins is an endothelial recycling pathway involving the neonatal Fc receptor (FcRn) expressed on endothelial cells. In the absence of the receptor, the concentration of immunoglobulins and albumin is decreased by one-third and the half-life is decreased to 1 day from 6 to 8 days.[31] This pathway is characterized by the uptake of these proteins by endocytosis and delivery to endosomes. Both immunoglobulin and albumin molecules bind to FcRn with high affinity at slightly acidic pH (below 6.5, as seen in endosomes) and consequently are protected from degradation. Endosomal contents following degradation are recycled into the blood at the apical plasma membrane or are transcytosed to the basolateral surface, where the neutral pH promotes the dissociation from FcRn and release into the interstitial fluid. The binding sites are different and saturable, and any excess of immunoglobulin that is not bound is degraded in the lysosomes.[7,27]

rFVIIIFc exploits this natural recycling pathway and is a novel Fc fusion protein that consists of a single (monomeric)

B-domain deleted FVIII molecule fused with an Fc dimer. The molecule has an extended half-life and was determined to be effective in preventing bleeding in preclinical studies. The protein is manufactured in the human embryonic kidney 293 (HEK293) cell line and has been extensively characterized.[32] rFVIIIFc is purified using a series of chromatography steps, including affinity capture with a recombinant single-chain antibody fragment, and no human or animal derived proteins are used in the purification or formulation processes. In addition, there are two dedicated viral clearance steps including a detergent treatment step for inactivation of capsulated viruses and a 15 nm filtration step for removal of viruses.

Clinical studies

An initial Phase I study evaluated the PK and safety of a single dose of rFVIIIFc,[33] which was followed closely by a Phase III study in adults[34] and children.[35] It is currently licensed for clinical use in the USA and Europe.

Adolescents and adults

The pivotal Phase III study (A-Long Study) was an open-label, multicenter, partially randomized study conducted in previously treated adolescents (>12 years) and adults with hemophilia A.[34]

It evaluated the safety and efficacy of repeated administration of rFVIIIFc for prophylaxis, treatment of acute bleeding, and prevention of bleeding during surgery. The study design took into account current regulatory requirements set by both the US Food and Drug Administration and European Medicines Agency.[36,37] One hundred and sixty-five patients were enrolled into three treatment arms: arm 1, an individualized prophylaxis arm that targeted prophylaxis to a minimum trough of 1%–3%; arm 2, once-weekly prophylaxis arm; and arm 3, an episodic arm that included on-demand treatment of bleeds. The primary efficacy end point was the comparison of per patient ABR in arm 1 versus arm 3. Another efficacy end point was the comparison of pharmacokinetic (PK) parameters of rFVIIIFc with rFVIII, and the rFVIII of choice was Advate® (antihemophilic factor [recombinant]) (Baxalta US Inc., Bannockburn, IL, USA), ie, rAHF-PFM (recombinant anti-hemophilic factor – protein-free medium). The primary safety end point was the development of neutralizing inhibitors confirmed by the Nijmegen-modified Bethesda assay (titer ≥0.6 BU/mL). Other secondary end points included the number of infusions and dose per infusion needed to treat a bleeding episode, and efficacy during surgical episodes.[34]

Patient recruitment across the study arms along with details of the prophylactic regimens are presented in Figures 1 and 2. Patients enrolled into the study were between the ages of 12 and 65 years and all ethnic groups were represented, with more than half being white Caucasian and a third being Asian. In keeping with the age range, a fifth were HIV
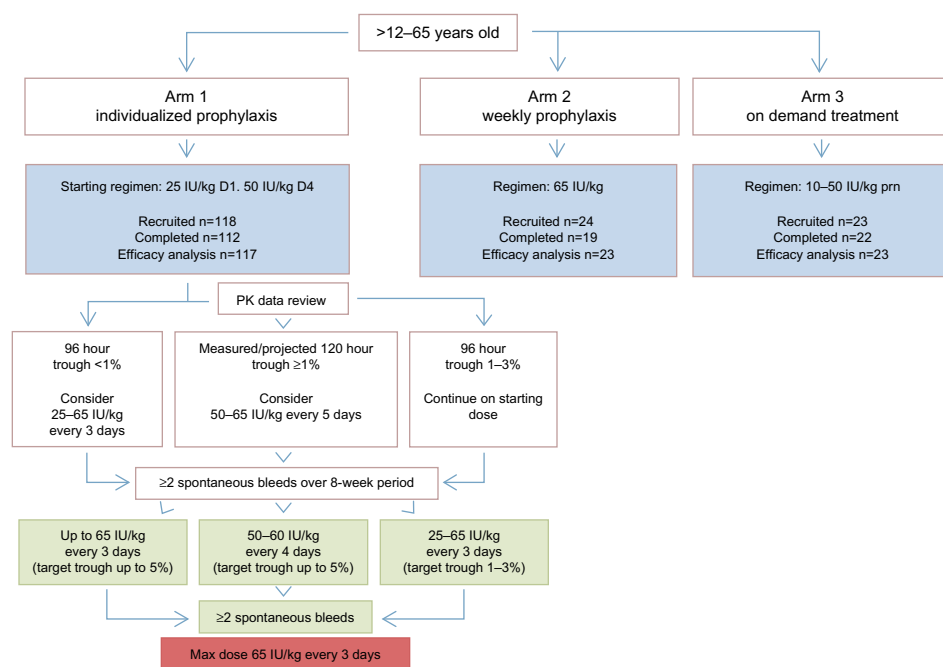

Figure 1 Study flowchart for adults and adolescents enrolled into A-Long studies.
Note: The disposition of patients in the trials along with details of dose adjustments that could be undertaken during the trial are shown.
Abbreviation: PK, pharmacokinetic.

Figure 2 Study flowchart for children enrolled into A-Long studies.
Note: The disposition of patients in the trials along with details of dose adjustments that could be undertaken during the trial are shown.
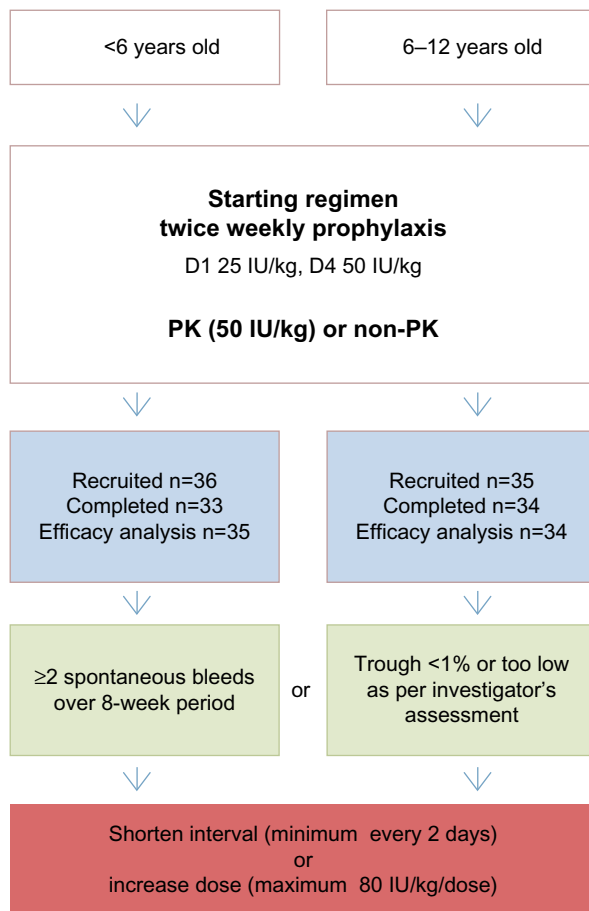**Abbreviation:** PK, pharmacokinetic.

positive, and about half the patient population had hepatitis C. Intron 22 inversion of the *F8* gene was the most common mutation present in the patient group, but all mutations were represented in the study population. The median and inter-quartile range (IQR) for von willebrand factor (VWF):Ag was 118.0 IU/dL (85, 153). Pretreatment ABR for 12 months before the study was estimated based on available data, and the median (IQR) for patients on prophylaxis was 6.0 (2, 15)

and 27.0 (17, 41) for patients receiving episodic treatment. The patient population is not dissimilar to patients involved in other pivotal studies, and an important exclusion criterion was the documented presence of a previous inhibitor even if it was not clinically relevant.

In arm 1, the starting treatment was a twice-weekly infusion using asymmetrical doses of 25 IU/kg on day 1 and 50 IU/kg on day 4. All patients at a minimum underwent an abbreviated PK study with some patients undergoing a full crossover PK study with rAHF-PFM. The details of the sampling time points are listed in Table 1. Following a PK analysis, dose and frequency adjustments were undertaken targeting replacement therapy to a minimum steady-state trough FVIII level of 1%–3%. The final treatment regimens included doses of 25–65 IU/kg administered at a frequency of 3–5 days. Dose adjustments were also undertaken if a patient experienced more than two spontaneous bleeds over an 8-week period, or if higher trough levels were desired to maintain good control of breakthrough bleeding.

Pediatric study

The rFVIIIfc fusion pediatric study (Kids–A Long) followed on from the pivotal adult and adolescent study and had a fairly standard approach for studies in previously treated children.[35] Only children with severe hemophilia (FVIII < 1%) and at least 50 exposure days were eligible, and documented presence of a previous inhibitor (or one detected at screening) was an exclusion criterion. A total of 71 boys were enrolled, with 67 completing the study, and the participants were divided into two cohorts of <6 and 6–12 years (both with equal number of participants). In the 6-12 year cohort all patients underwent PK study, and in the <6 years age group a limited number of children had a PK study performed. The primary end point was safety, including the development of neutralizing antibodies, and secondary end points included PK evaluation, per patient ABR, and the number of infusions needed to treat a bleeding episode. The dosing strategy was

Table 1 Sampling schedule used across different arms of the study

rFVIIIFc study	Arm/cohort	Dose of rFVIIIFc (IU/kg)	Number of patients	Sampling schedule
Phase I/IIa	Cohort A – crossover full PK[a]	25	6	0, 0.167, 0.5, 1, 3, 6, 9, 24, 48, 72, 96, 120, and 168 h
Phase I/IIa	Cohort B – crossover full PK[a]	65	10	As above +192, 216, and 240 h
Phase III	Arm 1 – crossover full PK[a]	50	28	0, 0.5, 1, 6, 24, 72, 96, and 120 h
Phase III	Arm 1 – abbreviated PK groups	50	155	0, 0.5, 3, 72, and 96 h
Pediatric study	Majority of patients	50	37	0, 0.5, 3, 24, 48, and 72 h

Notes: [a]The comparator for the crossover PK was rAHF-PFM (Advate®). The data for this table has been taken from published manuscripts related to Phase I, Phase III, and pediatric studies of rFVIIFc.[33–35,38] Adapted from Nestorov I, Neelakantan S, Ludden TM, et al. Population pharmacokinetics of recombinant factor VIII Fc fusion protein. *Clin Pharmacol Drug Dev.* 2015;4(3):163–174. © 2014, The American College of Clinical Pharmacology.[38]
Abbreviations: PK, pharmacokinetic; rFVIIIFc, recombinant FVIII Fc fusion protein; rAHF-PFM, recombinant anti-hemophilic factor – protein-free medium; h, hours.

asymmetrical and followed that of the pivotal study with a D1 dose of 25 IU/kg and a D4 dose of 50 IU/kg. Dose escalation or alteration of the dosing interval was allowed at the discretion of the treating physician. This could be either in response to breakthrough bleeds or in order to achieve a measurable FVIII trough level (>1%) if this was in keeping with local practice. Dose escalation to a total of 80 IU/kg/ per dose was allowable within the study design as was treating up to every 48 hours.

The study population not unexpectedly was diverse due to the multicenter nature of the study and wide variation in baseline clinical practice. While the majority of the study population had been on prophylaxis before enrolling (88.7%), the prophylaxis regimens varied significantly with 25.4% only being on once or twice a week infusions. The median estimated total number of bleeds in the 12 months before study enrollment was 2, but the range was from 0 to 36, which needs to be taken into account when interpreting the ABR data on the study.

Outcome – pharmacokinetics

Both single-dose PK and population PK models have been described for rFVIIIFc.[33,38] In the Phase I study, 16 patients underwent a PK study at two doses with antihemophilic factor (recombinant) as a comparator.[33] In the Phase III study, 28

subjects had a full PK study, with the remainder having an abbreviated PK study. In the pediatric study, of the 71 enrolled patients, 60 underwent a PK study and data are available from 54 patients. The dose and time points for the various PK studies are detailed in Table 1. A summary of the PK parameters by age group are listed in Table 2.

Incremental recovery was consistent between the age groups. The kinetics of rFVIIIFc activity display two-compartmental behavior, and the major covariate identified for clearance is the level of VWF:Ag. In the Phase III study, the rFVIIIFc half-life correlated with the baseline VWF:Ag level with a correlation coefficient of 0.67 (P<0.001).[34] PK analyses demonstrate reduced clearance of rFVIIIFc when compared to rAHF-PFM, although the volumes of distribution at steady state are very similar. A population PK model for rFVIIIFc has been described, and the covariates used for the model include age, weight, hematocrit, and VWF antigen level.[38] The PK data in these studies show that while there is a mean increase in the half-life of rFVIIIFc, demonstrating a 1.51 [1.38, 1.64]-fold increase in $t\frac{1}{2}$ (geometric mean ratio [95% CI]) in comparison to rAHF-PFM, the wide variability seen in the full-length factor VIII continues to be seen, and this is reflected by the wide confidence intervals. Furthermore, the study confirmed the findings of previous pediatric FVIII studies and the experience of clinicians treating children with severe hemophilia A, in that

Table 2 PK results of rFVIIIFc (efraloctocog alfa, Eloctate®) and rAHF-PFM (Advate®)

Parameter	rFVIIIFc – pediatric study		rFVIIIFc – Phase III pivotal study, abbreviated PK arm		rFVIIIFc – Phase III full crossover PK	rAHF-PFM – Phase III full crossover PK	rAHF-PFM pivotal study
Age (years)	2 to <6	6 to <12	12–17	≥18	≥12	≥12	10–65
Number of patients (n)	10	27	11	144	28	28	30
IR (IU/dL per IU/kg)	1.9 (1.7, 2.0)	2.4 (2.0, 2.9)	1.8 (1.6, 2.1)	2.0 (1.9, 2.1)	2.3 (2.1, 2.4)	2.35 (2.2, 2.5)	2.4±0.5
$t_{1/2}$ (h)	12.0 (9.5, 14.4)	14.6 (11.5, 17.7)	16.4 (14.1, 18.6)	17.8 (16.9, 18.7)	19.7 (17, 22)	12.4 (11.1, 13.9)	12.0±4.3
MRT (h)	16.4 (13.0, 19.7)	21.1 (16.8, 25.5)	23.1 (19.9, 26.4)	25.3 (24.1, 26.6)	26.1 (23, 29)	16.8 (15.2, 18.6)	15.69±6.21
CL (mL/h/kg)	3.9 (2.9, 4.8)	2.7 (2.3, 3.1)	2.7 (2.3, 3.0)	2.5 (2.3, 2.6)	2.06 (1.8, 2.3)	3.04 (2.7, 3.4)	3.0±1.0
V_{ss} mL/kg	58.7(54.7, 62.6)	49.9 (44.4, 55.3)	60.3 (32.3, 67.3)	57.5 (55.4, 59.5)	49.1 (46.6, 51.7)	51.2 (47.2, 55.5)	47.0±1.0
Mean relative change in $t_{1/2}$ (h)[a]	−33%	−18%	−8%	100%	NA	NA	NA
Mean relative change in CL (mL/h/kg)[a]	+58%	+9%	+8%	100%	NA	NA	NA

Notes: Data presented as mean (95% CI) and the Advate® data from pivotal studies are presented as mean ± SD. Data have been taken from published Phase I, Phase III, and pediatric studies and US FDA submission[33–35,39] and rounded off to the nearest decimal for simplicity. [a]The mean relative change compared to adult values (10%). Adapted with permission of American Society of Hematology, from Safety and prolonged activity of recombinant factor VIII Fc fusion protein in hemophilia A patients. Powell JS, Josephson NC, Quon D, et al. *Blood*. 2012;119(13):3031–3037; permission conveyed through Copyright Clearance Center, Inc.[33] Adapted with permission of American Society of Hematology, from Phase 3 study of recombinant factor VIII Fc fusion protein in severe hemophilia A. Mahlangu J, Powell JS, Ragni MV, et al. *Blood*. 2014;123(3):317–325; permission conveyed through Copyright Clearance Center, Inc.[34] Adapted from Young G, Mahlangu J, Kulkarni R, et al. Recombinant factor VIII Fc fusion protein for the prevention and treatment of bleeding in children with severe hemophilia A. *J Thromb Haemost*. 2015;13(6):967–977. © 2015 Young et al. Journal of Thrombosis and Haemostasis published by Wiley Periodicals, Inc. on behalf of International Society on Thrombosis and Haemostasis.[35] Adapted from Tarantino MD, Collins PW, Hay CR, et al. Clinical evaluation of an advanced category antihaemophilic factor prepared using a plasma/albumin-free method: pharmacokinetics, efficacy, and safety in previously treated patients with haemophilia A. *Haemophilia*. 2004;10(5):428–437. With permission from John Wiley and Sons.[39]
Abbreviations: PK, pharmacokinetics; IR, incremental recovery, the% rise in activity seen with an infusion of 1 IU/Kg of factor concentrate; $t_{1/2}$, terminal half-life; MRT, mean residence time; CL, systemic clearance; V_{ss}, volume of distribution at steady state; NA, not available; rFVIIIFc, recombinant FVIII Fc fusion protein; rAHF-PFM, recombinant anti-hemophilic factor – protein-free medium; SD, standard deviation; CI, confidence interval.

the younger the child the shorter the half-life and greater the clearance. Indeed in the pediatric study, in which a variety of prestudy FVIII treatments were used, the ratio of $t\frac{1}{2}$ of rFVII-IFc versus prestudy FVIII products ranged from 0.87 to 3.12 (1.22–2.11 in subjects receiving rAHF-PFM as prestudy FVIII), and this is shown in Figure 3. This is unlikely to be as wide in the case of adults but does emphasize once again the marked interindividual variability in the factor VIII half-lives. It also appears that the maximum improvement in half-life is likely to be seen in patients with higher levels of VWF: Ag and patients with a higher baseline $t\frac{1}{2}$.

Outcomes – efficacy
Prophylaxis

For the main study, a negative binomial regression model was used to calculate the ABR, and also, observed ABR data were provided.[34,35] The pediatric study provided data on observed ABR, and both studies provided ABR data for spontaneous, joint, and traumatic bleeds. The data were collected for a minimum of 6 months and extrapolated to 12 months. The ABRs by study arm and age category are listed in Table 3, and just under half of the patients did not have any bleeds during the trial period. Across both studies, ABRs were higher in patients with a higher number of target joints and a higher number of bleeds preceding study entry, while age and previous treatment had minimal impact on the ABR.

Management of bleeds

A single infusion was adequate for resolving the majority of bleeding episodes. In the pivotal study, 87.3% of bleeding episodes resolved with one injection and 97.8% of bleeding episodes required between one and two infusions.[34,35] Similarly in the pediatric study, 81.4% required a single infusion and 93.0% required between one and two infusions for resolution of bleeds. The median dose per infusion for resolution of a bleed was 49.7 IU kg in the pediatric study, while in the pivotal study it was 27.35 IU/kg. For bleeding episodes that required more than one infusion for resolution, the median (IQR) interval between the
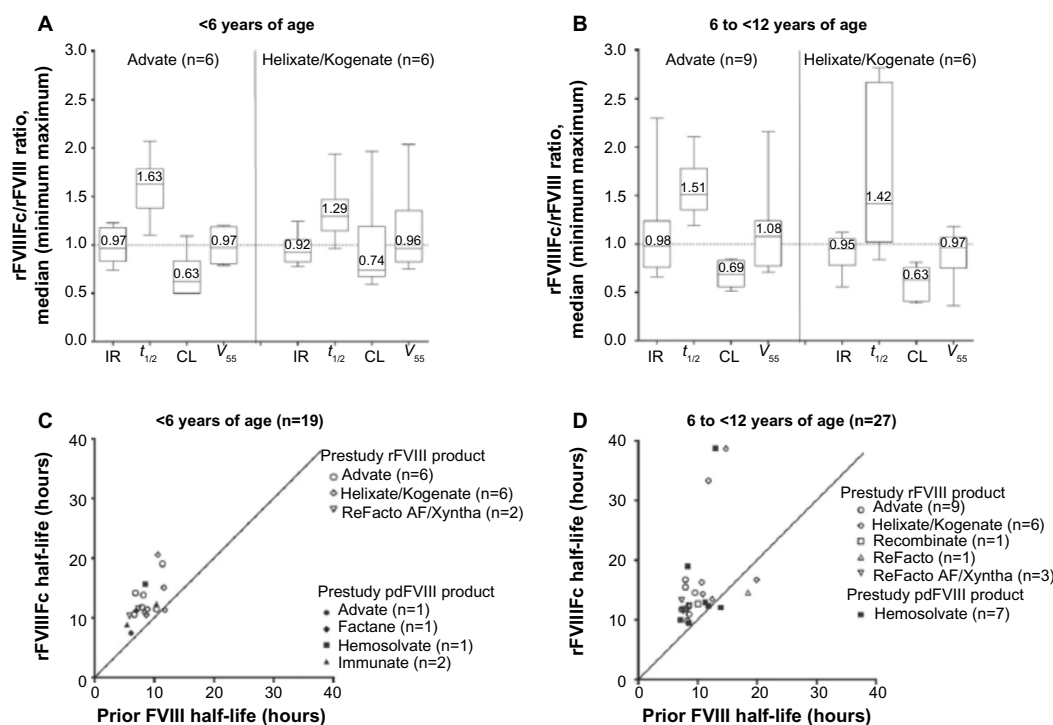

Figure 3 Comparison of rFVIIIFc and prestudy rFVIII PK parameters in pediatric study.
Notes: A comparison of the FVIII PK parameters for rFVIIIFc versus prestudy FVIII was performed for 46 subjects (<6 years of age, n=19; 6 to <12 years of age, n=27) with the one-stage clotting assay, and the ratio of 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® (antihemophilic factor [recombinant]) (**A**) or Helixate/Kogenate (**B**), are shown. Each 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 rFVIIIFc and prestudy FVIII PK was performed; data for the half-life comparison are shown by age cohort and prestudy FVIII product (**C, D**). Reproduced from Young G, Mahlangu J, Kulkarni R, et al. Recombinant factor VIII Fc fusion protein for the prevention and treatment of bleeding in children with severe hemophilia A. *J Thromb Haemost.* 2015;13(6):967–977. © 2015 Young et al. Journal of Thrombosis and Haemostasis published by Wiley Periodicals, Inc. on behalf of International Society on Thrombosis and Haemostasis.[35]
Abbreviations: PK, pharmacokinetic; rFVIIIFc, recombinant FVIII Fc fusion protein; CL, clearance; IR, incremental recovery; $t_{1/2}$, half-life; pdFVIII, plasma-derived FVIII; V_{ss}, volume of distribution at steady state.

Table 3 Key outcome data relating to bleeding episodes and weekly consumption of rFVIIIFc

Outcome	Pivotal study arm 1 N=117	Pivotal study arm 2 N=23	Pivotal study arm 3 N=23	Pediatric study 2 to <6 yr N=35	Pediatric study 6 to < 12 yr N=34
ABR, negative binomial model (95% CI)	2.9 (2.3–3.7)	8.9 (5.5–14.5)	37.3 (24.0–57.7)	NA	NA
Observed ABR[a] (all bleeds) Median (IQR) [range]	1.6 (0, 4.7)	3.6 (1.9, 8.4)	33.6 (21.1, 48.7)	0 (0, 3.9) [0–0.5]	2 (0, 4) [0–27.2]
Overall bleeds (spontaneous) Median (IQR) [range]	0 (0, 2)	1.9 (0, 4.8)	20.2 (12.2, 36.8)	0 (0, 0) [0.0–7.9]	0 (0, 0) [0.0–19.8]
Joint bleeds (spontaneous) Median (IQR) [range]	0 (0, 1.7)	0 (0, 3.8)	18.6 (7.6, 29.6)	0 (0, 0) [0.0–6.0]	0 (0, 0) [0.0–14.8]
Joint bleeds (traumatic) Median (IQR) [range]	0 (0, 1.2)	0 (0, 1.2)	3.9 (0.0, 8.6)	0 (0, 0) [0.0–6.5]	0 (0, 0) [0.0–7.9]
Muscle bleeds (spontaneous) Median (IQR)	0 (0, 0)	0 (0, 0)	5.1 (1.8, 6.8)	NA	NA
Muscle bleeds (traumatic) Median (IQR)	0 (0, 0)	0 (0, 0)	0 (0, 2)	NA	NA
Patients with no bleeding episodes, n (%)	53 (45%)	4 (17%)	0		46%
Weekly dose IU/kg, median (min, max for adults; IQR for pediatric patients)	77.9 (54.0, 141.5)	65.6 (59.4, 70.7)	NA	91.6 (84.7, 104.6)	86.9 (79.1, 103.1)

Notes: [a]In the extension study, the median (IQR) overall ABR in the arm 1 – 0.66 (0.22, 2.63), arm 2 – 2.03 (0.60, 4.39), 2–6 years group – 0.00 (0.00, 2.00), and 6–12 years group – 1.54 (0.00, 3.41).[42] Adapted with permission of American Society of Hematology, from Phase 3 study of recombinant factor VIII Fc fusion protein in severe hemophilia A. Mahlangu J, Powell JS, Ragni MV, et al. *Blood*. 2014;123(3):317–325; permission conveyed through Copyright Clearance Center, Inc.[34] Adapted from Young G, Mahlangu J, Kulkarni R, et al. Recombinant factor VIII Fc fusion protein for the prevention and treatment of bleeding in children with severe hemophilia A. *J Thromb Haemost*. 2015;13(6):967–977. © 2015 Young et al. Journal of Thrombosis and Haemostasis published by Wiley Periodicals, Inc. on behalf of International Society on Thrombosis and Haemostasis.[35] Adapted from Nolan B, Mahlangu J, Perry D, et al. Long-term safety and efficacy of recombinant factor VIII Fc fusion protein (rFVIIIFc) in subjects with haemophilia A. *Haemophilia*. 2016;22(1):72–80. © 2015 The Authors. Haemophilia Published by John Wiley & Sons Ltd.[42]
Abbreviations: rFVIIIFc, recombinant FVIII Fc fusion protein; ABR annualized bleed rate; CI, confidence interval; IQR, interquartile range; NA, not available; yr, years.

first and second infusion was 30.9 (24.5, 49.0) hours.[40] Because of the increased clearance seen in the age group of <6 years, it has been suggested that repeat dosing may be considered every 12–24 hours for moderate bleeding and every 8–24 hours for major bleeding,[41] although potentially this group is likely to tolerate a higher dose every 24 hours.

Management of surgical procedures

In the pivotal study, nine major surgical procedures were performed in nine individuals, with the majority of procedures being orthopedic procedures, including five knee joint replacements and one arthroscopy.[34] Other major procedures included laparoscopic hernia repair and appendectomy. The hemostatic control for all these procedures was rated excellent or good, and there were no concerns about hemostatic efficacy. The peak and trough levels aimed for during the procedures were in line with current international guidelines, with the majority being treated in line with levels used for developed countries. After a loading dose, repeat dosing could be given after 6–24 hours based on close monitoring of FVIII activity levels and type of surgery.[11,41] Similarly, minor procedures were undertaken within both the adult and pediatric studies with no concerns about hemostatic efficacy. It has been suggested that for minor surgery in adults a single dose may be adequate, but in children <6 years of age, repeat dosing after 12–24 hours may be considered.[11]

Outcome – safety
Neutralizing and Nonneutralizing antibodies

The safety data from the adult and pediatric study that included previously treated patients were reassuring but expected, with no evidence of neutralizing antibodies.[34,35]

No inhibitors have been reported during the extension study either.[42] The main study utilized the Nijmegen-modified Bethesda assay with a cut-off titer of ≥0.6 BU/mL for the test to be positive, and the pediatric study used the same assay with heat-inactivated specimens. The study also investigated for the presence of nonneutralizing antibodies (NNAs) by an electrochemiluminescence-based anti-rFVIIIFc binding antibody assay.

Five subjects from the pivotal study and seven children from the pediatric study tested positive for NNAs at baseline before administration of study drug and with no discernible problems.[34,35] In most instances, the NNA titers declined over the course of the study and were not detectable in two of five adult patients and four of seven pediatric patients at the completion of the study. De novo NNA antibody formation was documented in six adult patients, and in four of them the antibodies disappeared with time and in two patients they continued to be detected at the end of the study. None of the children were found to have developed de novo NNAs. NNAs were low titer, and none were directed against the Fc portion of the molecule. In one patient in arm 1, NNA presence was temporally associated with decreased recovery and increased clearance without any impact on the clinical outcome. It is well recognized that similar positive antibody tests are present in many individuals with severe hemophilia and indeed some normal controls, and their significance, if any, remains unclear.[43]

Other adverse events (AEs)

No thromboembolic events, hypersensitivity, or anaphylactic reactions were reported with drug administration.[34,35] The most common AEs reported by at least 5% of the patients were nasopharyngitis, arthralgia, headache, and upper

respiratory tract infection. Minor AEs that were considered possibly related to the study drug included headaches and arthralgias in one to two patients, and intermittent dark urine in one patient.

Laboratory monitoring

The assessment of postinfusion rFVIII levels in patients with hemophilia A in the majority of clinical laboratories is by one-stage clotting assay based on the activated partial thromboplastin time (APTT).[44] In recent years, there has been an increase in the number of clinical laboratories using chromogenic FVIII assays to measure postinfusion samples, though it is not in widespread use. Discrepancies between postinfusion FVIII monitoring by one-stage clotting factor and the chromogenic assay are well documented in the literature.[45–47] Critically when undertaking one-stage FVIII assays, the FVIII-deficient plasma used should contain VWF levels >30%.[48] When measuring BDD-rFVIII (B domain deleted, ReFacto®) by one-stage clotting factor assay, a product-specific standard is recommended.[47]

A recent field study across 30 laboratories worldwide compared three concentrations of rAHF-PFM (80, 20, and 5 IU/dL) with three concentrations of rFVIIIFc (87, 22, and 5.4 IU/dL) spiked into FVIII-deficient plasma.[49] The study demonstrated, as expected, higher recoveries (20%–30% higher) with the chromogenic assays than the one-stage clotting assays. The interlaboratory coefficient of variation (CVs) for both one-stage and chromogenic FVIII assays were variable (for spiked rAHF-PFM samples 10%–34% and 18%–38%, and for FVIIIFc 16%–37% and 19%–31%, respectively), with the lowest CVs for the 80% spiked samples and highest CVs for the 5% spiked samples, which was similar to a previous field study for another BDD-rFVIII.[46] As noted by the authors, a limitation of the rFVIIIFc field study was that spiked FVIII-deficient plasma was used and not patient samples, and therefore the study did not take into account patient factors that may influence the recovery level. This was partly

addressed in the Phase I study where postinfusion samples were analyzed by both one-stage clotting FVIII assay and chromogenic factor VIII assay, with the latter demonstrating higher recovery levels (21% higher in patients infused with rAHF-PFM versus 32% higher in patients infused with rFVIIIFc). It does appear that potentially either one-stage assay or chromogenic assay can be used without the need for a product-specific standard, although more studies are required to understand the true discrepancy between one-stage and chromogenic assays in real-world practice.

Translating into clinical use – adults

Data from the Phase I and pivotal study confirm the EHL and efficacy in prophylaxis. The extension in half-life is influenced by age and VWF levels, with younger patients showing a shorter half-life compared to older adults. The study confirms the beneficial effect of prophylaxis when compared to on-demand treatment and demonstrates the presence of a subgroup of patients who might benefit from once weekly prophylaxis. The individualization of prophylaxis with trough values of >1%, decreased the ABR compared to pretreatment ABR, and just under half of the patients in arm 1 had zero bleeds. Importantly for patients who continued into the extension study, the median ABR decreased in the individualized prophylaxis arm, suggesting the cumulative effect of prophylaxis on joint health and predisposition for bleeds. Table 4 lists the final treatment regimen at the end of pivotal study for 117 patients. Twenty-nine point one percent were treated twice weekly with an asymmetrical dosing regimen (25/50 IU/kg), and 29.1% of patients were treated every 5 days with 50 IU/kg. Seventeen point one percent of patients were treated with 50 IU/kg every 3 days, and the highest dose of 65 IU/kg every 3 days was used in 8.5% of the patients, and this group probably represents patients with a shorter half-life. Overall, patients were distributed approximately by thirds into twice weekly, every 3 days, and every 5 days dosing with fewer than 4% treated every 4 days.

Table 4 Final dosing schedule at end of Phase III pivotal study illustrating the interindividual variability in prophylactic dosing regimens[a]

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

Note: [a]Adapted with permission of American Society of Hematology, from Phase 3 study of recombinant factor VIII Fc fusion protein in severe hemophilia A. Mahlangu J, Powell JS, Ragni MV, et al. *Blood.* 2014;123(3):317–325; permission conveyed through Copyright Clearance Center, Inc.[34]
Abbreviation: rFVIIIFc, recombinant FVIII Fc fusion protein.

What does the increased half-life mean in practical terms? Patients may require an abbreviated PK study to determine their individual half-life on rFVIIIFc or any other EHL product to achieve the most benefit. The study has shown that decreased frequency of infusions with adequate trough levels is feasible, and patients with longer half-lives are able to take advantage of longer dosing intervals and decreased frequency of infusions. However, conceptually the most clinical benefit perhaps may be seen in patients with a shorter half-life where rFVIIIFc with slightly increased half-life may provide better coverage with a higher trough levels when administered at the same frequency as standard rFVIII as these individuals are particularly at risk of bleeding from a delayed or missed dose. Furthermore, there may be scope for some dose reduction. Clinically, in preference to performing PK studies (even if abbreviated), higher VWF:Ag levels may potentially be used as a surrogate marker for identifying patients who might benefit from EHL products in the context of strong correlation with half-life, but in the absence of stratified analysis it is difficult to define a threshold level of VWF:Ag that would identify patients with long or short half-life. The identification of VWF:Ag threshold while not perfect may aid decision making regarding dose and frequency of prophylaxis that would need to be closely followed by adjustments based on clinical monitoring with trough levels and bleed frequency. Pragmatically, if a patient already requires alternate day dosing, there may be scope to reduce the dose with an EHL product such as rFVIIIFc and still maintain adequate trough levels, or increase the interval to every 3 days or twice a week. Those patients who already need less frequent dosing will likely be able to extend this further, translating to one less infusion a week. It is important to note that the best outcomes in terms of ABR were observed in the twice weekly and every third-day regimens.

Translating into clinical use – children

The median ABR in the children's study was 1.96, similar to the results seen in the pivotal study with a prestudy-estimated median ABR of 2.00. Approximately 46% of enrolled patients did not report a bleed on the study. However, in a cohort with a wide range of prestudy ABRs and at least one individual with a high prestudy ABR of 36, not unsurprisingly some patients continued to have significant numbers of bleeding episodes while on the study. The heterogeneity of the population makes extrapolation of the trial data to clinical practice difficult, in that while the median spontaneous ABR was 0.00, there were boys with

a spontaneous ABR of >14 in the study, which might be considered a poor outcome for the individual patient in the context of the resources used.

The vast majority of bleeds resolved with one or two doses of rFVIIIFc (81.4% and 93%, respectively), with the majority of bleeds being reported as traumatic nonjoint bleeds. It is anticipated that in the context of major joint bleeds, treatment to complete resolution should continue to be the standard of care, and patients will require daily treatment, with more than one dose required on the first day, and frequency of infusion decreasing beyond the first day.

With regard to the infusion frequency, the study design stipulated two infusions in a 7-day period and at the end of study, 62 of 69 boys continued to remain on twice weekly infusions with seven patients requiring an increase in the frequency of their infusions. The median total weekly dose at the end of the study was 88.11 IU/kg (IQR 80.29–103.2), and the wide IQR, as suggested by the authors, may have related to a "rounding up effect" related to vial sizes. However, it is equally possible that larger doses were needed either to achieve measurable troughs out to 96 hours or to prevent spontaneous bleeding episodes in some of the younger cohort. In the context of a clinical trial, there is relative freedom to increase prophylaxis doses to maintain measurable FVIII troughs if that is the normal practice with standard half-life FVIII products while keeping the subject on a twice weekly regimen. However, once, licensed, it seems much more likely that rFVIIIFc will be used in children at a higher dose frequency than that outlined in the study design, after individual assessment of PK, even if this is in a limited form.

The use of EHL FVIII products in boys with severe hemophilia A will be an evolving process over the next 5–10 years as both clinicians and families become more familiar with them. A pragmatic approach will be necessary to manage both clinician and patient expectations, involving evaluation of the individual's response to EHL products before planning a permanent product switch or deciding on the precise nature of the subsequent prophylaxis regimen.

Conclusion

The licensing and availability on the market of EHL recombinant factor VIII concentrates such as Eloctate® has extended the opportunities for personalized hemophilia care. The trial has reconfirmed the benefit of prophylaxis and was both reassuring in terms of safety and efficacy for routine prophylaxis and management of bleeds and surgery. However, the protocols used within the context of a clinical

trial have taken into account a limited number of factors in designing the prophylaxis regimens, but modern hemophilia care needs to take into account all factors that contribute to a patient's individual bleeding phenotype and adherence and modify prophylaxis accordingly in order to reduce the burden of illness for the best possible outcomes. It may be that these products can be used at a conventional frequency in those patients with short half-lives to achieve a more satisfactory trough level or at decreased frequency in those with better half-lives, thus decreasing the burden of disease either through improved outcomes or better adherence to treatment. The recognition of the impact of interindividual variability in half-lives on outcomes is a major challenge to the hemophilia community, which needs to develop prophylaxis algorithms with surrogate markers in addition to the clinical outcomes.

Disclosure

PC has served on advisory boards for Biogen Idec and Sobi Biovitrum and has served as an investigator in rFVIIIfc studies. MM has served as an investigator in rFVIIIfc studies. EF and AR report no conflicts of interest in this work.

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