



## Long-lasting recombinant factor VIII proteins for hemophilia A

Amy D. Shapiro<sup>1,2</sup>

<sup>1</sup>Indiana Hemophilia and Thrombosis Center, Indianapolis, IN; and <sup>2</sup>Department of Pediatrics, Michigan State University, East Lansing, MI

In the past 50 years, the lifespan of an individual affected with severe hemophilia A has increased from a mere 20 years to near that of the general unaffected population. These advances are the result of and parallel advances in the development and manufacture of replacement therapies. We are now poised to witness further technologic leaps with the development of longer-lasting replacement therapies, some of which are likely to be approved for market shortly. Prophylactic therapy is currently the standard of care for young children with severe hemophilia A, yet requires frequent infusion to achieve optimal results. Longer-lasting products will transform our ability to deliver prophylaxis, especially in very young children. Longer-lasting replacement therapies will require changes to our current treatment plans including those for acute bleeding, prophylaxis, surgical interventions, and even perhaps immunotolerance induction. Ongoing observation will be required to determine the full clinical impact of this new class of products.

### Background

Before the advent of efficient replacement therapy, the life expectancy of a child with severe hemophilia A was approximately 20 years.<sup>1,2</sup> Currently, the life expectancy of this population is near that of the general unaffected population<sup>2,3</sup>—a major achievement unparalleled in other chronic genetic conditions. The first recombinant factor VIII (rFVIII) concentrates were approved in the United States in 1992.<sup>4</sup> rFVIII concentrates have improved the product supply, decreased to near zero viral bloodborne infections, and allowed the development of prophylactic treatment regimens. Now, 21 years later, a new class of rFVIII concentrates are emerging that will allow us to challenge our current treatment regimens and paradigms of care. Sequelae of hemophilia include musculoskeletal complications, development of inhibitory antibodies, and transmission of viral bloodborne infections.<sup>5,6</sup> Uncontrolled or unrecognized major bleeding events may have severe consequences, including local functional deficits, hemorrhagic shock, neurocognitive defects, and death.<sup>7,8</sup> These significant events may be minimized by the use of prophylactic treatment regimens.<sup>9</sup>

Prophylactic treatment regimens achieve a less severe deficiency based upon nadir FVIII levels accomplished with regularly scheduled infusions. Treatment regimens to achieve optimal bleed suppression and prevention vary individually; some patients tolerate nadir levels  $\leq 1\%$ , whereas others require higher nadir levels to achieve the desired therapeutic outcome.<sup>10,11</sup> Current standard prophylactic regimens commonly use infusion therapy administered 3 times weekly, whereas other regimens require every other day administration; regimens are individually modified to achieve optimal bleed suppression yet tailored to the patient's age and individual and family needs.

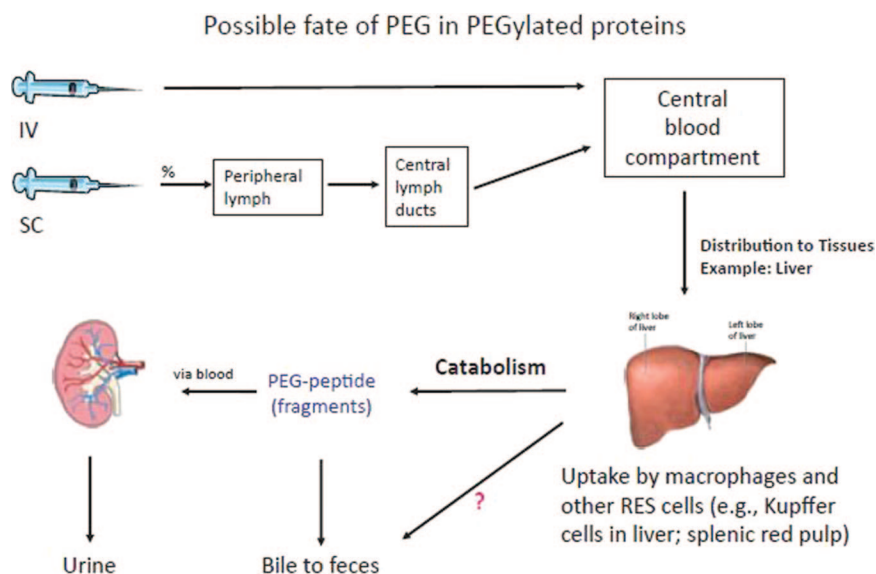
Chief among the associated issues with current regimens is the need for adequate venous access and patient/family compliance. These issues are magnified in the very young pediatric population, in whom central venous access devices (CVADs) have been used to overcome technical difficulties. Although CVADs make prophylaxis feasible in young children, they are associated with complications, most notably mechanical failure including fracture, dehiscence of the skin over the reservoir, infection, and thrombosis. Once

placed and used, families may have difficulty with CVAD removal and transition to peripheral venous access.<sup>12</sup>

Adherence to demanding therapeutic regimens that include frequent morning infusion to achieve adequate hemostatic coverage during periods of highest activity make these regimens less effective and compromise their cost-benefit ratio.<sup>13</sup> In addition, significant health-care provider efforts are required to ensure adequate comprehension of the rationale for and requirements of the therapeutic plan, follow-up to detect compliance barriers, and development of agreed-upon solutions to overcome identified issues.

As children progress into adolescence, adherence is often further compromised.<sup>14</sup> Decreased frequency of required infusion and the achievement of higher trough levels could affect this age group's ability to fully use and adhere to therapeutic regimens and achieve improved outcomes. In addition, the use of prophylactic regimens have therapeutic advantages even in adults with prior musculoskeletal sequelae including decreased numbers of acute hemorrhagic episodes, slowed progression of joint disease in previously affected areas, prevention of joint disease in previously unaffected areas, and improved quality of life and productivity.<sup>15,16</sup> The use of prophylaxis in adults with severe hemophilia A is inconsistent and not standardized. The advent of longer-acting products may increase the likelihood that affected adults choose this therapeutic option and enjoy improved outcomes.

The development of inhibitory alloantibodies (inhibitors) occurs in  $\sim 25\%$  to  $30\%$  of severe hemophilia A patients and in  $3\%$  to  $13\%$  of those with moderate or mild disease.<sup>17,18</sup> Inhibitors neutralize infused FVIII and therefore greatly affect the ability of the patient to achieve hemostasis. The use of bypassing agents is required for the treatment of bleeding episodes, and the effective use of prophylaxis is greatly diminished in this segment of the population. Much attention has been aimed at improved algorithms to predict inhibitor development and the development of regimens to modify the individual's immune response.<sup>19</sup> Patients with inhibitors are fragile and require considerable management expertise. The use of bypassing agents and/or immunotolerance regimens is exceedingly costly. Products with decreased immunogenicity coupled with improved



**Figure 1. Removal of PEG in PEGylated proteins administered either IV or subcutaneously.** The PEGylated protein is likely removed through the mechanisms specific to the protein, assuming that this takes place in the liver. After degradation of the likely more labile protein part, the PEG molecule remains mostly intact because PEG metabolism is limited. PEG molecules may be excreted mainly by the kidney, but to some extent also through bile.<sup>24</sup> Reprinted with permission from Ivens et al.<sup>24</sup> Copyright 2012, Blackwell Publishing Ltd.

predictive methods would therefore be desirable if they are able to affect inhibitor development.

Therefore, despite major therapeutic advances in the treatment of hemophilia A, opportunities clearly remain to optimize and transform therapy.

### Background of long-acting FVIII proteins

A variety of methodologies have been applied to achieve longer-lasting FVIII products. Initial attempts to combine FVIII with pegylated liposomes did not yield a prolonged half-life ( $T_{1/2}$ ).<sup>20</sup> Specific FVIII modifications to either increase its activity<sup>21</sup> or decrease proteolytic degradation<sup>22,23</sup> have been attempted, although these specific constructs were not effective in preclinical models and thus did not enter the clinical setting. The greatest success to date has been achieved with either site-specific or controlled covalent attachment to polyethylene glycol (PEG) or the production of fusion proteins with either a monomeric Fc fragment of immunoglobulin G<sub>1</sub> or albumin. PEG protects FVIII against proteolytic degradation, whereas fusion technology uses alternative recycling pathways to diminish the impact of natural proteolytic mechanisms for FVIII clearance. These 2 general approaches will be described in greater detail below.

### Technology of long-acting FVIII proteins

PEGylation may improve pharmacokinetics (PK), pharmacodynamics, and immunological profiles and is a well-established technology that has current approved therapeutics in variety of disease states.<sup>24</sup> The use of PEG creates a hydrophilic cloud around FVIII and inhibits its proteolytic degradation while allowing its normal function of binding to VWF and participation in coagulation once activated. FVIII clearance is partly attributed to specific binding to the low-density lipoprotein receptor-related protein (LRP1), a hepatic clearance receptor with broad specificity. FVIII  $T_{1/2}$  can be prolonged in mice when LRP1 is blocked.<sup>25</sup> PEG interferes with binding to LRP1 and is likely important in the prolongation of FVIII  $T_{1/2}$ .

The rate of clearance of PEG FVIII is related to PEG size, with smaller molecules more rapidly cleared.<sup>24</sup> Clinical trials with these agents appear to retain desired FVIII properties including binding to VWF while not raising specific clinical concerns. The clearance of PEG, once injected, especially on a regular basis as required for prophylaxis in hemophilia A, has generated discussion and analysis.<sup>25</sup> The physiologic site for PEG clearance (eg, renal or liver) is largely dependent on its size (Figure 1). To date, significant PEG-associated adverse events in preclinical studies or clinical trials include a rare occurrence of hypersensitivity reactions. Long-term potential PEG-associated clinical effects, when regularly injected IV, also may relate to the PEG size, PEG number bound to each molecule, and overall yearly calculated PEG load. Ongoing clinical trials with a wider patient base and long-term follow-up are required; however, analyses from currently licensed products using PEG indicate a reasonable safety margin given current yearly PEG exposure estimates.<sup>24</sup> There is some evidence that PEG conjugation may affect antigen presentation of FVIII, resulting in decreased antigenicity; the translation of this observation to impact of inhibitor development would be significant but awaits further clinical trials. For a discussion on this topic, please refer to the chapter by Kaufman and Powell in this publication entitled “Molecular Approaches for Improved Clotting Factors for Hemophilia.”<sup>26</sup>

Fusion proteins to either albumin via a linker<sup>27</sup> or to a monomeric Fc fragment of immunoglobulin G<sub>1</sub><sup>28</sup> take advantage of natural pathways to prolong FVIII  $T_{1/2}$ ; specifically the neonatal Fc-receptor, which results in pH-dependent recycling within endosomes to the plasma membrane, prolonging its  $T_{1/2}$ <sup>29</sup> (Figure 2). Although fusion via a linker to albumin for rFIX is under clinical study, the FVIII albumin fusion construct was not pursued.

### Preclinical and clinical studies of long-acting FVIII proteins

Three different rFVIII PEG conjugates are currently in clinical development: B-domain deleted rFVIII (PEG-BDD-rFVIII; BAY 94-9027) has completed phase 1 ([www.clinicaltrials.gov](http://www.clinicaltrials.gov) identifier

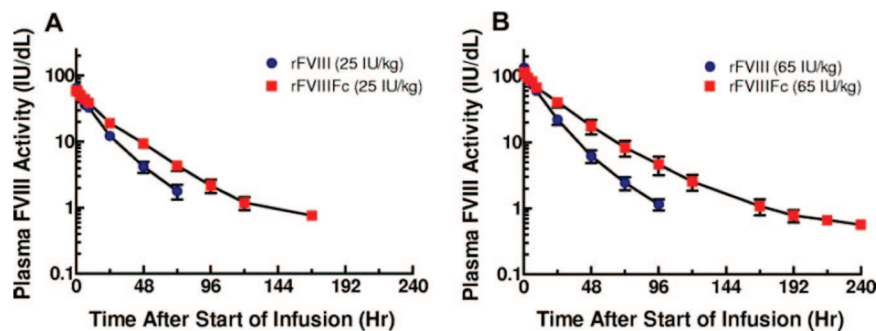


Figure 2. Group mean plasma FVIII activity PK profiles for low- and high-dose cohorts.<sup>34</sup> Reprinted with permission from Powell et al.<sup>34</sup> Copyright 2012, American Society of Hematology.

NCT01184820); phase 3 studies are ongoing for PEGylated full-length rFVIII (BAX 855; www.clinicaltrials.gov identifier NCT01736475) and glyco-PEGylated rFVIII (N8-GP; www.clinicaltrials.gov identifier NCT01480180). These products differ in the length of the FVIII molecule, the cell line in which they are produced, the strategy to combine with PEG, and the size of the PEG used (Table 1).

N8-GP is a rFVIII with site-directed glycoPEGylation that prolongs  $T_{1/2}$  while preserving hemostatic activity.<sup>35</sup> The rFVIII (N8) is synthesized in a Chinese hamster ovary (CHO) cell line and has a truncated B-domain of 21 amino acids with demonstrated PK properties similar to those of other current therapies.<sup>36</sup> The terminal sialic acid on an O-glycan structure in the truncated B-domain is replaced by a conjugated sialic acid containing a branched 40-kDa PEG that results in a protein with a single PEG attached to the B-domain. Upon thrombin activation, the B domain is cleaved with the attached PEG, leaving activated FVIII.

The safety and PK of N8-GP was evaluated in patients and compared with their previously used rFVIII product. This dose escalation study (25, 50, or 75 IU/kg/dose) included 26 previously treated patients (PTPs) with severe FVIII. N8-GP was well tolerated at all doses; no patients developed an inhibitor or binding antibodies to rFVIII or N8-GP. The PK of N8-GP was dose linear with a mean terminal  $T_{1/2}$  of 19 hours (range, 11.6-27.3 hours), representing a 1.6-fold increase over prior products. Clearance was reduced by 30% and the volumes of distributions were similar compared with the patients' comparator products. This product is currently in phase 3 clinical trials.

The most common method to attach PEG is via an attachment to lysine residues or N-terminal amines present in the native protein. Attachment through these mechanisms may lead to inhibition of normal protein function via interference with normal target receptors and binding interactions. Controlled PEG site binding is critical to maintain function and produces a homogeneous, consistent product. Site-specific PEGylation can be accomplished through site-specific mutagenesis that introduces cysteine mutations on the surface of BDD-FVIII. BAY94-9027 was engineered with surface-exposed cysteines to which PEG was conjugated with retention of full in vitro activity and VWF binding. Improved PK was demonstrated in mice and rabbits. PK studies in VWF knock-out mice revealed the larger rFVIII PEG molecule may substitute for VWF to protect the protein from in vivo clearance. The PEGylated rFVIII exhibited prolonged efficacy consistent with improved PK and demonstrated effectiveness in acute bleeding in hemophilic mice.<sup>25</sup> A phase 1 study in humans using this agent was recently completed

and revealed that it was well tolerated, efficacious, and without serious adverse events. Phase 2/3 studies are ongoing.<sup>24</sup> An 8-week prospective, multicenter, open-label nonrandomized phase 1 study in PTPs with severe hemophilia A without inhibitors administered 2 cohorts of 7 patients either 25 IU/kg/dose twice weekly or 60 IU/kg/dose once weekly of BAY 94-9027. The product demonstrated improved PK parameters, with a  $T_{1/2}$  of ~19 hours compared with the comparator non-Pegylated product, and was well tolerated without immunogenicity.<sup>30</sup>

BAX 855 is a 20 kDa PEGylated full-length rFVIII. The conjugation process yields 2 moles PEG per FVIII molecule with 60% of PEG chains localized to the B-domain. The PEGylation process is based upon reaction of an activated PEG reagent with accessible amino groups on FVIII and optimized so that mainly the  $\epsilon$ -amino groups of lysine residues are targeted and modified. This process does not compromise specific activity and is controlled for production consistency. BAX 855 is reported to retain all physiologic properties of FVIII except binding to the LRP clearance receptor.

Preclinical testing was performed in standard animal models, including FVIII-deficient dogs, knock-out mice, and macaques, and revealed normal activity and prolonged  $T_{1/2}$  compared with unmodified rFVIII.<sup>31</sup>

Using Fc fusion technology, rFVIII Fc was developed by fusing a single B-domain-deleted rFVIII, to the dimeric Fc region of IgG<sub>1</sub> without intervening linker sequences.<sup>28,37,38</sup> rFVIII Fc is produced from human embryonic kidney (HEK) 293 cells via recombinant DNA technology. HEK cells ensure fully human, posttranslational modifications,<sup>39-42</sup> including glycosylation, with theoretical functional relevance compared with nonhuman mammalian expression systems such as CHO cells, which insert nonhuman glycosylations. Glycosylation modulates yield, bioactivity, solubility, stability against proteolysis, immunogenicity, and clearance rate from circulation.<sup>43</sup> Because CHO cells produce glycans at the N-terminal position that differ from human glycans, there is the potential for an increased risk of immunogenicity in humans.<sup>43</sup> The biochemical and functional characterization of rFVIII Fc compared with existing FVIII products demonstrated that rFVIII Fc maintains normal interactions with proteins necessary for activity, and with prolonged in vivo activity resulting from fusion with Fc.<sup>37</sup> The posttranslational modifications of rFVIII Fc were similar to other rFVIII molecules.<sup>37</sup> The binding of rFVIII Fc to VWF was comparable to that of other rFVIII molecules.<sup>37</sup> rFVIII Fc specific activity showed that the function of the FVIII moiety of rFVIII Fc was not compromised as a result of the Fc fusion.<sup>37</sup>

**Table 1. Long-acting rFVIII clotting factors**

Product	Manufacturer	Technology	FVIII	Cell line	Modification	Mean T <sub>1/2</sub>	T <sub>1/2</sub> × native FVIII	Clinical trial
Bay 94-9027 <sup>24,25,30</sup>	Bayer HealthCare	Site-specific PEGylation	B domain deleted	BHK21	Branched 60kDa PEG	18.2 h	~1.4	Phase 2/3, ongoing
Bax 855 <sup>31</sup>	Baxter	PEGylation by controlled chemical means at specific lysine residues	Full-length	CHO	20 kDa PEG	~18* h	1.5	Phase 1 completed; phase 2/3 ongoing
NB-G <sup>32,33</sup>	Novo Nordisk	Single-site-specific glycoPEGylation	B domain truncated (21 aa)	CHO	40 kDa PEG modified sialic acid	18 h	~1.6	Phase 2/3, ongoing
rFVIII-Fc <sup>34</sup>	Biogen Idec	Fusion protein with monomeric Fc fragment IgG <sub>1</sub>	B domain deleted	HEK 293 human cell line†	Fusion to Fc fragment of IgG <sub>1</sub>	18.8 h	1.54-1.7	Phase 3 ongoing and completed; under regulatory review

\*Extrapolated T<sub>1/2</sub> from Advate data.

†Hybrid cell line of HEK293 and a human B-cell line.

That the neonatal Fc receptor (FcRn) is responsible for the prolonged T<sub>1/2</sub> of rFVIII-Fc was also confirmed; the T<sub>1/2</sub> of rFVIII-Fc was approximately 2-fold higher than that of rFVIII in hemophilia A mice, hemophilia A dogs, normal mice, and transgenic mice expressing human FcRn. In contrast, the T<sub>1/2</sub> of rFVIII-Fc was comparable to rFVIII in FcRn knock-out mice, confirming that the interaction between FcRn and the Fc fragment is responsible for conferring protection of the Fc fusion protein from degradation.<sup>38</sup>

### PK of rFVIII-Fc

In the first-in-human phase 1/2a study (www.ClinicalTrials.gov identifier NCT01027377), 16 PTPs with severe hemophilia A (< 1% FVIII activity) received a single dose of either 25 or 65 IU/kg rFVIII followed by an equal dose of rFVIII-Fc.<sup>34</sup> rFVIII-Fc showed an ~ 1.5- to 1.7-fold increase in mean T<sub>1/2</sub> (18.8 hours for both 25 and 65 IU/kg rFVIII-Fc vs 12.2 hours for 25 IU/kg rFVIII and 11.0 hours for 65 IU/kg rFVIII; *P* < .001 for both comparisons).<sup>34</sup> rFVIII-Fc showed a 30% to 35% reduction in clearance compared with rFVIII. rFVIII and rFVIII-Fc had comparable dose-dependent peak plasma concentrations and recoveries (Figure 3).

Interestingly, individual VWF levels correlated well with both rFVIII-Fc clearance (*R*<sup>2</sup> = 0.5492, *P* = .0016) and T<sub>1/2</sub> (*R*<sup>2</sup> = 0.6403, *P* = .0003). An important observation is that Fc fusion technology does not prolong the T<sub>1/2</sub> of rFVIII-Fc and rFIX-Fc to the same extent, one potential explanation being the association of rFVIII-Fc with VWF. Unlike FIX, ~ 98% of FVIII circulates in complex with VWF.<sup>44</sup> The approximate 18-hour T<sub>1/2</sub> of VWF<sup>45</sup> is thought to limit the degree to which the T<sub>1/2</sub> of rFVIII products can be extended,<sup>25,34,38</sup> which is consistent with the observed T<sub>1/2</sub> of rFVIII-Fc in the phase 1/2a study and the reported T<sub>1/2</sub> with PEG-modified FVIII products in trial (Table 1).

### Clinical safety of rFVIII-Fc

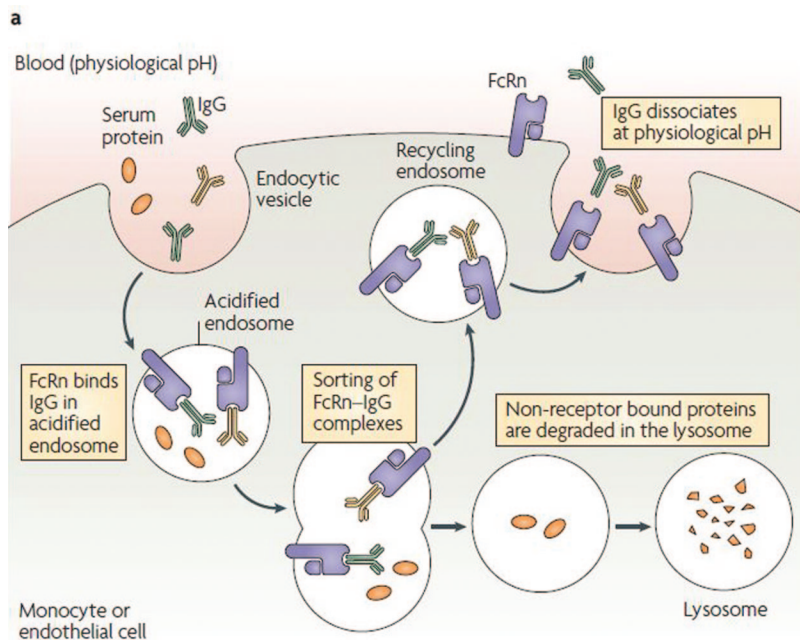
In the phase 1/2a study in 16 PTPs, rFVIII-Fc was well tolerated at both 25 and 65 IU/kg.<sup>34</sup> No drug-related serious adverse events were observed. No serious bleeding episodes were observed and, importantly, no inhibitors, non-neutralizing antibodies, or allergic reactions were reported.

### Clinical development of rFVIII-Fc

#### Pivotal phase 3 study

A-LONG, an open-label, multicenter, phase 3 study designed to evaluate the safety, PK, and efficacy of rFVIII-Fc in PTPs with severe hemophilia A (www.ClinicalTrials.gov identifier NCT01181128), was recently completed. A-LONG enrolled patients ≥ 12 years of age into 3 study arms: (1) individualized prophylaxis (3- to 5-day intervals), (2) weekly prophylaxis, and (3) episodic (on-demand) treatment. Patients from each treatment arm were eligible to enter a surgery subgroup. The primary safety end point was the development of inhibitors and the primary efficacy end point for A-LONG was the annualized bleeding rate. PK of rFVIII-Fc versus rFVIII (Advate), number of injections required for bleed control, and perioperative hemostasis were evaluated.

The dosing regimens investigated in A-LONG, once published, will provide valuable information on the impact that rFVIII-Fc may have on current hemophilia care. Although the individualized prophylaxis arm enabled patients to extend their dosing interval, data from the weekly prophylaxis arm will provide insight into how this fixed



**Figure 3. FcRn recycling pathway.**<sup>29</sup> IgG and Fc fusion proteins are taken up from circulation into cells by nonspecific pinocytosis and/or endocytosis mechanisms.<sup>49</sup> As the endosomes become acidic, the Fc domain of IgG or Fc fusion proteins binds to FcRn. Once the endosome fuses back at the cell surface, Fc dissociates from FcRn at neutral pH and IgG and Fc fusion proteins are released back into the circulation.<sup>49</sup> In contrast, circulating proteins that do not interact with FcRn are trafficked to endosomal and lysosomal degradation pathways.<sup>29,50</sup> Ultimately, Fc degrades naturally and does not accumulate in the body.<sup>51</sup> Despite its name, the expression of human FcRn is stable throughout life.<sup>52</sup> Reprinted with permission from Roopenian and Akilesh.<sup>29</sup> Copyright 2007, Nature Publishing Group.

regimen may potentially provide a more appealing option for individuals who currently receive episodic treatment. A weekly regimen could lead to a change in attitude in such individuals and guide them toward individualized prophylaxis and its associated benefits.

#### Ongoing rFVIIIc studies

Because many hemophilia patients are children, further study in the pediatric population is important. The Kids A-LONG study ([www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) identifier NCT01458106) is currently recruiting ~ 50 children < 12 years of age who have severe hemophilia A and have had at least 50 documented prior exposures to FVIII. The study is designed to evaluate the prophylactic use of rFVIIIc for the control and prevention of bleeding in previously treated children. The frequency of inhibitor development is the primary outcome measure and annualized bleeding rate and response to treatment for bleeding episodes are secondary outcome measures. PK parameters are particularly important to assess in the pediatric population because they may differ relative to patient age or body weight. Indeed, rFVIII clearance has been shown to decline and  $T_{1/2}$  to increase with age<sup>46</sup> and it has been suggested, based on PK studies, that the dose required to maintain a desired trough level during prophylaxis may therefore require adjustment every 2 years or when significant weight gain occurs.<sup>47</sup>

Patients who complete the A-LONG and Kids A-LONG studies have the option to continue treatment in the extension study, ASPIRE ([www.clinicaltrials.gov](http://www.clinicaltrials.gov) identifier NCT01454739). ASPIRE is evaluating the long-term safety and efficacy of rFVIIIc and has 2 treatment regimen options: prophylaxis and episodic treatment.

#### Conclusions

Many practical hurdles are associated with current therapeutic regimens for treating hemophilia. The relatively short  $T_{1/2}$  of available coagulation factor products necessitate frequent infusions, with recurrent venipuncture often leading to CVAD use in young children, which in turn is associated with a risk for thrombosis, infection, and mechanical failures. Current regimens aim to maintain trough levels at or above 1% between doses. Nonetheless, optimal treatment regimens for hemophilia have yet to be defined; some patients bleed despite having a trough level above 1%, whereas others having undetectable trough levels do not experience breakthrough bleeding. Peak levels have been demonstrated to be important in the development of breakthrough bleeding and require further evaluation.<sup>48</sup>

The advent of long-acting products represents an important advance in the management of hemophilia, with the potential to change current care paradigms. Long-lasting products could provide the opportunity for prolonged protection from bleeding and bleed resolution with fewer injections. Institution of early prophylaxis with once weekly infusion through peripheral venipuncture may be feasible in very young children. The need for CVADs and their associated risks could decrease. Less burdensome regimens using long-acting products offer the potential for enhanced protection with increased adherence and, therefore, improved long-term outcomes. Patients on episodic treatment may be encouraged to transition to a prophylaxis regimen to improve joint outcomes and ultimately quality of life. Long-acting coagulation factors provide an opportunity to target higher trough levels and improved individualized treatment, resulting in increased protection from bleeding episodes. It is currently accepted that higher trough levels may be needed for some patients or in specific circumstances (eg, for

participation in sports and activities). However, our current paradigm for target trough levels in prophylaxis regimens approximating 1% may be reconsidered and challenged with the advent of longer-lasting replacement therapies. Ease of measurement of these newer agents with currently available standardized assays will allow our current treatment protocols to change over time without significant difficulty. Long-acting factors have the potential to substantially improve acute management of bleeds, markedly simplify prophylactic regimens, and provide an opportunity for improved individualized treatment for hemophilia A. How these products will affect the current cost of therapy is as yet unknown.

## Acknowledgments

Special thanks to Natalie Duncan for her excellent assistance.

## Disclosures

Conflict-of-interest disclosure: The author declares no competing financial interests. Off-label drug use: None disclosed.

## Correspondence

Amy D. Shapiro, MD, Medical Director, Indiana Hemophilia & Thrombosis Center, 8402 Harcourt Rd, Suite 500, Indianapolis, IN 46260; Phone: 317-871-0000; Fax: 317-871-0010; e-mail: ashapiro@ihtc.org.

## References

- Larsson SA. Life expectancy of Swedish haemophiliacs, 1831-1980. *Br J Haematol*. 1985;59:593-602.
- Plug I, Van Der Bom JG, Peters M, et al. Mortality and causes of death in patients with hemophilia, 1992-2001: a prospective cohort study. *J Thromb Haemost*. 2006;4:510-516.
- Darby S, Kan S, Spooner R, et al. Mortality rates, life expectancy, and causes of death in people with hemophilia A or B in the United Kingdom who were not infected with HIV. *Blood*. 2007;110:815-825.
- Kingdon HS, Lundblad RL. An adventure in biotechnology: the development of haemophilia A therapeutics - from whole-blood transfusion to recombinant DNA to gene therapy. *Biotechnol Appl Biochem*. 2002;35:141-148.
- Srivastava A, Brewer A, Mauser-Bunschoten EP, et al. Guidelines for the management of hemophilia. *Haemophilia*. 2013;19:e1-e47.
- Berntorp E, Shapiro AD. Modern haemophilia care. *Lancet*. 2012;379:1447-1456.
- Simpson ML, Valentino LA. Management of joint bleeding in hemophilia. *Expert Rev Hematol*. 2012;5:459-468.
- Kauvar DS, Lefering R, Wade CE. Impact of hemorrhage on trauma outcome: an overview of epidemiology, clinical presentations, and therapeutic considerations. *J Trauma*. 2006;60:S3-S11.
- Manco-Johnson MJ, Abshire TC, Shapiro AD, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med*. 2007;357:535-544.
- Ahnström J, Berntorp E, Lindvall K, Björkman S. A 6-year follow-up of dosing, coagulation factor levels and bleedings in relation to joint status in the prophylactic treatment of haemophilia. *Haemophilia*. 2004;10:689-697.
- Collins PW, Björkman S, Fischer K, et al. Factor VIII requirement to maintain a target plasma level in the prophylactic treatment of severe hemophilia A: influences of variance in pharmacokinetics and treatment regimens. *J Thromb Haemost*. 2010;8:269-275.
- Ewenstein BM, Valentino LA, Journeycake JM, et al. Consensus recommendations for use of central venous access devices in haemophilia. *Haemophilia*. 2004;10:629-648.
- Thornburg C. Prophylactic factor infusions for patients with hemophilia: challenges with treatment adherence. *JCD*. 2009;1-6.
- Schrijvers LH, Uitslager N, Schuurmans MJ, Fischer K. Barriers and motivators of adherence to prophylactic treatment in haemophilia: a systematic review. *Haemophilia*. 2013;19:355-361.
- Aledort LM, Haschmeyer RH, Pettersson H. A longitudinal study of orthopaedic outcomes for severe factor-VIII-deficient haemophiliacs. The Orthopaedic Outcome Study Group. *J Intern Med*. 1994;236:391-399.
- Tagliaferri A, Franchini M, Coppola A, et al. Effects of secondary prophylaxis started in adolescent and adult haemophiliacs. *Haemophilia*. 2008;14:945-951.
- World Federation of Hemophilia. Diagnosis and management of inhibitors to factors VIII and IX: an introductory discussion for physicians. Available from: <http://www1.wfh.org/publication/files/pdf-1178.pdf>. Accessed May 30, 2013.
- Kempton CL, Allen G, Hord J, et al. Eradication of factor VIII inhibitors in patients with mild and moderate hemophilia A. *Am J Hematol*. 2012;87:933-936.
- Gouw S, van der Bom J, van den Berg H, CANAL Study Group. Treatment-related risk factors of inhibitor development in previously untreated patients with hemophilia A: the CANAL cohort study. *Blood*. 2007;109:4648-4654.
- Powell JS. Liposomal approach towards the development of a longer-acting factor VIII. *Haemophilia*. 2007;13:23-28.
- Kaufman RJ, Pipe SW. Can we improve on nature? "Super molecules" of factor VIII. *Haemophilia*. 1998;4:370-379.
- Gale AJ, Pellequer JL. An engineered interdomain disulfide bond stabilizes human blood coagulation factor VIIIa. *J Thromb Haemost*. 2003;1:1966.
- Gale AJ, Radtke KP, Cunningham MA, Chamberlain D, Pellequer JL, Griffin JH. Intrinsic stability and functional properties of disulfide bond-stabilized coagulation factor VIIIa variants. *J Thromb Haemost*. 2006;4:1315-1322.
- Ivens IA, Baumann A, McDonald TA, Humphries TJ, Michaels LA, Mathew P. PEGylated therapeutic proteins for haemophilia treatment: a review for haemophilia caregivers. *Haemophilia*. 2013;19:11-20.
- Mei B, Pan C, Jiang H, et al. Rational design of a fully active, long-acting PEGylated factor VIII for hemophilia A treatment. *Blood*. 2010;116:270-279.
- Kaufman RJ, Powell JS. Molecular approaches for improved clotting factors for hemophilia. *Hematology Am Soc Hematol Educ Program*. 2013;2013:30-36.
- Metzner HJ, Weimer T, Kronthaler U, Lang W, Schulte S. Genetic fusion to albumin improves the pharmacokinetic properties of factor IX. *Thromb Haemost*. 2009;102:634-644.
- Peters RT, Low SC, Kamphaus GD, et al. Prolonged activity of factor IX as a monomeric Fc fusion protein. *Blood*. 2010;115:2057-2064.
- Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. *Nat Rev Immunol*. 2007;7:715-725.
- Coyle T, Reding M, Lin J, Michaels L, Shah A, Powell J. Abstract FP-MO-03.2-3: An open-label phase I study to evaluate the pharmacokinetics and safety profile of Bay 94-9027, a PEGylated B-domain-deleted recombinant factor VIII, in previously treatment patients with severe hemophilia A. *Haemophilia*. 2012;18(Suppl 3):22.

31. Turecek PL, Bossard MJ, Graninger M, et al. BAX 855, a PEGylated rFVIII product with prolonged half-life: development, functional and structural characterisation. *Haemostaseologie*. 2012;32(Suppl 1):S29-S38.
32. Tiede A, Brand B, Fischer R, et al. Enhancing the pharmacokinetic properties of recombinant factor VIII: first in-human trial of glycoPEGylated recombinant factor VIII in patients with hemophilia A. *J Thromb Haemost*. 2013;11:670-678.
33. Thim L, Vandahl B, Karlsson J, et al. Purification and characterization of a new recombinant factor VIII (N8). *Haemophilia*. 2010;16:349-359.
34. Powell JS, Josephson NC, Quon D, et al. Safety and prolonged activity of recombinant factor VIII Fc fusion protein in hemophilia A patients. *Blood*. 2012;119:3031-3037.
35. Agerso H, Stennicke HR, Pelzer H, et al. Pharmacokinetics and pharmacodynamics of turoctocog alfa and N8-GP in haemophilia A dogs. *Haemophilia*. 2012;18:941-947.
36. Martinowitz U, Bjerre J, Brand B, et al. Bioequivalence between two serum-free recombinant factor VIII preparations (N8 and ADVATE®) – an open-label, sequential dosing pharmacokinetic study in patients with severe haemophilia A. *Haemophilia*. 2011;17:854-859.
37. Peters RT, Toby G, Lu Q, et al. Biochemical and functional characterization of a recombinant monomeric factor VIII-Fc fusion protein. *J Thromb Haemost*. 2013;11:132-141.
38. Dumont JA, Liu T, Low SC, et al. Prolonged activity of a recombinant factor VIII-Fc fusion protein in hemophilia A mice and dogs. *Blood*. 2012;119:3024-3030.
39. Durocher Y, Butler M. Expression systems for therapeutic glycoprotein production. *Curr Opin Biotechnol*. 2009;20:700-707.
40. Walsh G. Post-translational modifications of protein biopharmaceuticals. *Drug Discov Today*. 2010;15:773-780.
41. Swiech K, Picanco-Castro V, Covas DT. Human cells: new platform for recombinant therapeutic protein production. *Protein Expr Purif*. 2012;84:147-153.
42. Zhang M, Mezo A. Enzymatic release and detection of galactose-alpha-1,3-galactose (a-Gal) in recombinant FVIII products. *Haemophilia*. 2012;18(Suppl 3):PO-MO-135.
43. Ghaderi D, Zhang M, Hurtado-Ziola N, Varki A. Production platforms for biotherapeutic glycoproteins. occurrence, impact, and challenges of non-human sialylation. *Biotechnol Genet Eng Rev*. 2012;28:147-176.
44. Lenting PJ, CJ VANS, Denis CV. Clearance mechanisms of von Willebrand factor and factor VIII. *J Thromb Haemost*. 2007;5:1353-1360.
45. Suiter T, Laffan M, Mannucci PM, et al. Recombinant human von Willebrand factor (rVWF): First-in-human study evaluating pharmacokinetics, demonstrating safety and tolerability in type 3 von Willebrand disease [abstract]. *Blood (ASH Annual Meeting Abstracts)*. 2010;116(21):237.
46. Bjorkman S, Oh M, Spotts G, et al. Population pharmacokinetics of recombinant factor VIII: the relationships of pharmacokinetics to age and body weight. *Blood*. 2012;119:612-618.
47. Ljung R, Auerswald G, Benson G, et al. Novel coagulation factor concentrates: Issues relating to their clinical implementation and pharmacokinetic assessment for optimal prophylaxis in haemophilia patients. *Haemophilia*. 2013;19(4):481-486.
48. Lindvall K, Astermark J, Björkman S, et al. Daily dosing prophylaxis for haemophilia: a randomized crossover pilot study evaluating feasibility and efficacy. *Haemophilia*. 2012;18:855-859.
49. Lencer WI, Blumberg RS. A passionate kiss, then run: exocytosis and recycling of IgG by FcRn. *Trends Cell Biol*. 2005;15:5-9.
50. Ghetie V, Ward ES. Multiple roles for the major histocompatibility complex class I- related receptor FcRn. *Annu Rev Immunol*. 2000;18:739-766.
51. McGarry T, Hough R, Rogers S, Rechsteiner M. Intracellular distribution and degradation of immunoglobulin G and immunoglobulin G fragments injected into HeLa cells. *J Cell Biol*. 1983;96:338-346.
52. Israel EJ, Taylor S, Wu Z, et al. Expression of the neonatal Fc receptor, FcRn, on human intestinal epithelial cells. *Immunology*. 1997;92:69-74.