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Extended Half-Life Factor VIII and Factor IX Preparations

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Summary
In the last couple of years, several extended half-life factor VIII and factor IX preparations were intensively studied and gained approval. In order to extend half-lives, techniques like fusion to protein conjugates (Fc part of IgG_1 or albumin), chemical modification (PEGylation), and protein sequence modification are implemented. With these techniques, it is possible to extend half-lives of factor IX products 4- to 6- fold, while half-life extension of factor VIII products is limited to 1.5- to 2-fold due to their interaction with von Willebrand factor. Nevertheless, both extended half-life factor VIII and IX products have improved and facilitated prophylactic factor replacement therapy in hemophilia A and B, respectively. Extended half-life factor concentrates pose challenges to coagulation laboratories because accurate therapy monitoring is not possible with all factor activity assays currently used.

Introduction

Hemophilia A and B are X-linked bleeding disorders resulting from deficiencies of coagulation factors VIII (FVIII) and IX (FIX), respectively [1]. Hemophilia A occurs in 1 of 5,000 and hemophilia B in 1 of 30,000 male live births [1].

Modern treatment of hemophilia A and B started in the 1950s and early 1960s when it was possible to treat bleeding episodes with infusions of whole blood and fresh plasma. But only the detection of cryoprecipitate in 1964 by Judith Graham Pool allowed it to infuse enough FVIII in relative small volumes to effectively control bleeding and to make surgery possible in a safe way [2]. Further development resulting in production of FVIII concentrates and prothrombin complex factors (containing FIX) that could be reconstituted in small amounts of fluid allowed home treatment and led to the introduction of prophylactic factor replacement schedules [3, 4]. Today, both plasma-derived factor concentrates and genetically engineered recombinant factor concentrates enable hemophilia treatment without the risk of transmission of infectious agents [6].

The efficacy of prophylactic treatment in preventing hemophilic arthropathy was retrospectively described by Nilsson and colleagues [4] in a milestone publication in 1992 and later formally confirmed in a randomized controlled trial by Manco-Johnson and collaborators [5].

Nowadays, the important issues in hemophilia treatment remain the risk of development of neutralizing alloantibodies (inhibitors; occurring in up to 40% of patients with severe hemophilia A and in about 3% of patients with severe hemophilia B) [6] and a considerable high treatment burden for patients due to the need of periodic intravenous injections to maintain FVIII or FIX levels in a safe range (usually FVIII or FIX levels > 1%).

FVIII has a half-life of 12 h with high inter-individual variability [7]. Therefore, most patients with severe hemophilia A need to inject themselves three times a week or more often with conventional FVIII concentrates in a prophylactic setting [8]. Half-life of FIX is slightly longer (18–24 h) [7] but patients with severe hemophilia B still need to inject themselves twice a week on average with FIX concentrates to be protected from spontaneous bleeding [8]. Despite these demanding prophylactic treatment schedules, patients with severe forms of hemophilia still have an elevated risk of bleeding and are not fully protected from spontaneous bleeding episodes [9–12]. Taken together, there is a need for safer, longer acting, and more convenient methods to treat patients with hemophilia A or B [13]. In the last years, modifications of FVIII and FIX aiming at the extension of the half-life of these molecules have been in the focus of product development [14]. Several of these extended half-life (EHL) products have meanwhile gained approval by regulatory bodies and are available to patients.

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Table 1. Extended half-life FVIII products. Mean half-lives are given for patients > 12 years only[a]

Generic name	Name	Technology	Cell line	Molecule length	Mean half-life, h
Efmoroctocog alfa	Elocta®	Fc-fusion	HEK	BDD	19
Rurioctacog alfa pegol	Adynovi®	PEGylation to surface exposed lysine	CHO	full-length	14–16
Turoctocog alfa pegol	–	single site-specific PEGylation to O-linked glycan in B-domain	CHO	BD truncated	19
Damoctocog alfa pegol	–	site-specific PEGylation to cysteine1805	BHK	full-length	19
Lonoctocog alfa	Afstyla®	covalently linked heavy and light chain with increased affinity for vWF	CHO	BDD	14.5

HEK = Human embryonic kidney; CHO = Chinese hamster ovary; BHK = baby hamster kidney; vWF = von Willebrand Faktor; BDD = B-domain deleted; BD = B-domain.
[a]Note that methods to evaluate half-lives differed among studies.

Methods to Extend Half-Lives

Fusion to Protein Conjugates

Both Immunoglobulin G (IgG) and albumin are proteins with a very long half-life of up to several weeks [15]. Both proteins undergo an intracellular recycle mechanism via the neonatal Fc receptor (FcRn), which protects them from lysosomal degradation [16, 17]. After internalization by the endothelial cell, proteins bound to the FcRn receptor in the acidified endosome are protected from lysosomal sorting and degradation. This allows recycling back to the cell surface with pH-dependent release into circulation [18].

This recycling mechanism is still in place when the Fc-part of IgG (fc fusion) or albumin (albumin fusion) is directly fused to clotting factors such as FVIII and FIX leading to extended in vivo half-lives of the fused proteins [16].

Chemical Modification

Covalent modification of therapeutic proteins with polyethylene glycol (PEG) chains is an established approach to prolong half-life and in vivo activity of proteins [19]. Linking one or more PEG chains to a therapeutic molecule is also known as PEGylation. The PEG molecule is non-immunogenic, non-toxic, and highly hydrophilic. PEG conjugation increases the circulation time of FVIII and FIX mainly by protecting against enzymatic digestion and blocking interaction with clearance receptors [20].

Protein Sequence Modification

Human FVIII consists of two glycoprotein chains and circulates in plasma in a complex with von Willebrand factor (vWF). The molecular weight of such a full-length FVIII is approximately 280 kDa; it is composed of a heavy chain (domains A1a1 A2a2B) and a light chain (domains a3A3C1C2), which are held together by non-covalent interactions [21].

In contrast to other commercially available FVIII concentrates that are two-chains molecules, a recombinant FVIII single chain (rFVIII-SingleChain) has been developed. This single-chain preparation consists of a truncated B-domain that covalently links the heavy and light chains. As the three predominant thrombin cleavage sites are not affected in this concept, activation by thrombin leads to a structurally normal activated FVIII molecule. rFVIII-SingleChain shows improved intrinsic stability and a markedly increased affinity to vWF. This higher affinity to vWF leads to improved pharmacokinetic properties in terms of a prolonged half-life [22].

Limitations of Prolonging Half-Life of FVIII

In human plasma, the vast majority (95–98%) of FVIII is complexed to vWF. The interaction between vWF and FVIII is crucial for FVIII function, immunogenicity, and clearance, because vWF is essentially serving as chaperone for FVIII. Clearance of vWF and FVIII occurs mostly as a complex leading to a half-life of FVIII of approximately 12 h. Bioengineered FVIII variants such as PEGylated forms, Fc-part of IgG-coupled forms but also rFVIII-SingleChain are still regulated to a large extent by interaction with vWF. Therefore, half-life of vWF (approximately 15 h, high variability between individuals) is the limiting factor to half-life extension of FVIII with techniques available today. All approaches described above achieve only moderate increases of half-lives (1.5- to 2-fold compared to unmodified FVIII) [23].

This is in contrast to FIX where it was possible to increase the mean half-life 4- to 6-fold by different forms of bioengineering [24–26].

Extended Half-Life Factor Products

FVIII Products (Table 1)

Fc-Fused FVIII

A single molecule of a B-domain-deleted recombinant FVIII (rFVIII; human cell line) is covalently fused to the Fc domain of IgG_1 (rFVIIIFc or efmoroctocog alfa). Efmoroctocog alfa (marketed as Elocta® in Europe) has been evaluated for safety, efficacy,

and pharmacokinetics in two pivotal phase 3 studies [27, 28]. The first included previously treated patients (PTPs) with severe hemophilia A ≥ 12 years and had three treatment arms: individualized prophylaxis (arm 1, n = 118), weekly prophylaxis (arm 2, n = 24), and episodic treatment (arm 3, n = 23) [27]. Terminal half-life of rFVIIIFc was extended 1.5-fold as compared to non-EHL rFVIII. rFVIII was tolerated well, resulted in fairly low annualized bleeding rates (ABRs) of 1.6 (interquartile range (IQR) 0.0–4.7; arm 1) and 3.6 (IQR 1.9–8.4; arm 2), and reduced dosing frequency in the majority of patients in arm 1 as compared to their previous treatment schedules. None of the subjects developed an inhibitor.

The second phase 3 study included 71 previously treated children aged < 12 years with severe hemophilia [28]. The starting rFVIIIFc regimen was twice weekly prophylaxis that was adjusted as needed. No subject developed an inhibitor, and rFVIIIFc half-life was prolonged relative to that of FVIII 1.29- to 1.63-fold (dependent on the individual pre-study FVIII product). Overall median ABRs were 1.96 (IQR 0.00–3.96).

Interim data of an rFVIIIFc extension study confirm both long-term safety and maintenance of low ABRs with extended interval prophylactic dosing [29].

PEGylated FVIII

Several PEGylated FVIII products have been developed. They differ from each other by the PEGylation sites and the molecule length of FVIII.

Rurioctacog alfa pegol (BAX855; Adynovi®) is created through controlled PEGylation of a full-length, unmodified rFVIII (synthesized in Chinese hamster ovary cells), in which approximately 60% of PEG chains are localized at the B-domain. BAX855 was evaluated in 138 PTPs with severe hemophilia A aged 12–65 years [30]. The mean residence time of BAX855 compared to rFVIII (Advate) was prolonged 1.4- to 1.5-fold. In a prophylaxis schedule (45 IU/kg, 2×/week, n = 120) ABR was 1.9 (IQR 0.00–5.80), and 39.6% of compliant subjects had no bleeding events during prophylaxis.

A second study evaluated BAX855 in 73 PTPs < 12 years in a prophylactic setting with twice weekly infusions [31]. No subject developed FVIII inhibitors, and ABR was 3.04 (95% CI 2.21–4.19; joint bleedings 1.1 (0.64–1.91), spontaneous bleedings 1.16 (0.74–1.83)).

Furthermore, it was shown that BAX855 is safe and hemostatically effective in patients with severe hemophilia A undergoing surgery [32].

N8-GP (turoctocog alfa pegol) contains a B-domain-truncated rFVIII synthesized in Chinese hamster ovary cells. PEGylation is reached by replacing the terminal sialic acid on an O-glycan structure in the truncated B-domain by a conjugated sialic acid containing a branched 40-kDa PEG. This way of PEGylation does not affect hemostatic activity [33]. N8-GP has been evaluated in a phase 3 trial including 186 PTPs ≥ 12 years with severe hemophilia A [33]. Patients were allocated to receive N8-GP for prophylaxis (50 IU/kg every 4 days; n = 175) or on demand treatment. Mean terminal half-life of 8-GP was 1.6-fold longer when compared to standard rFVIII. In the prophylaxis arm the median ABR was 1.33 (IQR 0.00–4.61) while 40% of patients had no bleeding events during the trial phase. One patient developed low-titer inhibitory antibodies against FVIII after 93 exposure days. After another 3 months of treatment inhibitor titer increased markedly, and the patient was withdrawn from the trial.

In a pediatric cohort (68 PTPs < 12 years), N8-GP was tested in a twice weekly prophylactic regimen [34]. More than 40% of patients did not report any bleeding during trial phase and reported median ABR was 1.95 (IQR 0.00–2.79). Half-life ratio between N8-GP and patients' previous FVIII product was 1.85. None of the pediatric patients developed inhibitors. N8-GP has not been marketed yet.

BAY 94-9027 (damoctcog alfa pegol) is a B-domain-deleted (BDD) rFVIII (baby hamster kidney cell line) that conjugates in a site-specific manner to a single 60-kDa PEG molecule at an engineered cysteine [35]. In a pivotal trial (phase 2/3 study) 134 PTPs ≥ 12 years with severe hemophilia A received BAY 94-9027 for 36 weeks on either an on demand setting or prophylactically (n = 112; intervals determined individually following a 10-week run-in period on 25 IU/kg twice weekly) [36]. 43 of 112 patients on prophylaxis were assigned to a once-weekly schedule (60 IU/kg) after the run-in period. 32 of these 43 patients (74%) who continued every-7-days prophylaxis until study end, had a low median ABR of only 0.96 (IQR 0.00–4.30). BAY94-9027 is not yet available on the market, and the study program is ongoing.

rFVIII-SingleChain

The single chain product lonoctocog alfa (Afstyla®) has been evaluated for efficacy and safety in two pivotal trials. The first trial [37] included PTPs with severe hemophilia A ≥ 12 years. Participants were allocated to receive either on-demand (n = 27) or prophylactic (n = 146) treatment with rFVIII-SingleChain. Across all prophylaxis regimens, median spontaneous ABR was 0.0 (IQR 0.0–2.4), and median overall ABR was 1.14 (IQR 0.0–4.3). No participant developed a clinically relevant inhibitor during the study phase. Investigators rated hemostasis as excellent or good in all 16 surgical procedures.

In a second trial [38], 84 boys (all PTPs) with severe hemophilia < 12 years were included. Again, patients were allocated to either prophylactic (n = 81) or on-demand treatment. Median ABR for spontaneous bleeding was 0.0 (0.00–2.20) while it was 3.69 (0.00–7.20) for all bleedings across all prophylaxis regimens. Hemostatic efficacy was rated at least good in the vast majority of cases, and none of the participants developed an inhibitor.

Lonoctocog alfa was compared in a pharmacokinetic study [39] against a full-length rFVIII (octocog alfa; Advate®) and revealed a slightly longer mean half-life (14.5 vs. 13.3 h) and a larger mean area under the curve (AUC; 35% larger than octocog alfa).

For the sake of completeness, it has to be mentioned that other preparations such as the human cell line-derived recombinant FVIII (human-cl rhFVIII; Nuwiq®) also showed slightly increased half-lives due to high affinity for vWF [40]. However, these products are usually not considered to be EHL factors because they are neither chemically modified nor fused to other proteins.

Table 2. Extended half-life FIX products. Mean half-lives are given for patients > 12 years only[a]

Generic name	Name	Technology	Cell line	Molecule length	Mean half-life, h
Eftrenanocog alfa	Alprolix®	Fc-fusion	HEK	full-length	82
Albutrepenonacog alfa	Idelvion®	albumin-fusion	CHO	full-length	102
Nonacog beta pegol	Refixia®	site-specific PEGylation to activation peptide	CHO	full-length	93

HEK = Human embryonic kidney; CHO = Chinese hamster ovary.
[a]Note that methods to evaluate half-lives differed among studies.

FIX Products (Table 2)

Fc-Fused FIX

rFIX-Fc (eftrenonacog alfa; Alprolix®) was the first EHL getting approval by a regulatory body (US Food and Drug Administration (FDA)) in March 2014. Eftrenonacog alfa is composed of a single recombinant FIX molecule (human cell line) fused to the dimeric Fc domain of IgG_1 [24]. In a phase 3 study with 123 PTPs with severe hemophilia B ≥ 12 years [24], rFIX-Fc was tested in 4 treatment groups: group 1 (n = 63) received weekly dose-adjusted prophylaxis, group 2 (n = 29) received interval-adjusted prophylaxis, group 3 (n = 27) received on-demand treatment, and group 4 received perioperative treatment. In the pharmacokinetics subgroup rFIX-Fc exhibited a prolonged terminal half-life of 82 h (half-life of conventional FIX: 17 h). Median ABRs were 3.0 (IQR 1.0–4.4) in group 1, 1.4 (IQR 0.0–3.4) in group 2, and 17.7 (IQR 10.8–23.2) in group 3. Hemostasis was rated as excellent or good in 14/14 major surgeries. No inhibitors have been detected. In a second phase 3 study, pediatric PTPs (n = 30) with severe hemophilia B < 12 years have been included [41]. All patients were initially given rFIX-Fc prophylaxis once per week, with adjustment to dose or dosing frequency as needed. In this population, rFIX showed a prolonged half-life of 69 h. Median ABR was 2.0 (IQR 0.0–3.1) for all bleeding episodes and 0.0 (IQR 0.0–0.0) for joint bleedings. 29 of 30 patients remained on once-weekly infusions during the study period, and none of the participants developed an inhibitor.

Albumin-Fused FIX

Albutrepenonacog alfa (rFIX-FP; Idelvion®) is a recombinant fusion protein linking recombinant FIX (Chinese hamster ovary cell line) with recombinant albumin. A cleavable linker between FIX and albumin is derived from the endogenous activation peptide in native FIX [25]. Similar to other products, rFIX-FP was evaluated in a phase 3 study with 63 PTPs with severe hemophilia B ≥ 12 years [25]. The study included 2 groups: group 1 (n = 40) started with a once-weekly prophylaxis schedule for 26 weeks followed by either 7-, 10-, or 14-day prophylaxis regimen; group 2 (n = 23) patients received on-demand treatment for 26 weeks and then switched to a once-weekly prophylaxis regimen. In pharmacokinetic assessment, rFIX-FP showed a half-life of 102 h. Median ABR for spontaneous bleeds was 0.0 (IQR 0.00–0.96) for all prophylaxis regimens. There was a 100% reduction of target joint bleedings when subjects switched from on-demand to prophylaxis treatment. None of the study subjects developed an inhibitor.

A second phase 3 study included 27 male PTPs with severe to moderately severe hemophilia B < 12 years [42]. All boys received routine prophylaxis with rFIX-FP once weekly. Terminal half-life of rFIX-FP was 91.4 h in this pediatric population (4.3-fold longer than with previous FIX products). Median ABR for spontaneous bleedings was 0.0 (IQR 0.00–0.91), and patients maintained a median trough level of 13.4 IU/dl. No inhibitors have been detected.

19 patients participating in one of the mentioned phase 3 studies investigating rFIX-Fc underwent 21 surgical procedures. In 20/21 surgeries, a single preoperative dose maintained intraoperative hemostasis, and hemostasis was rated excellent or good in all procedures [43].

PEGylated FIX

Nonacog beta pegol (N9-GP; Refixia®) is a recombinant FIX product (Chinese hamster ovary cell line) linked to 40 kDa PEG moiety (attached to the FIX activation peptide) [26]. In a phase 3 study, 74 PTPs > 12 years with severe to moderately severe hemophilia B were randomized to either 10 (n = 30) or 40 IU/kg (n = 29) once-weekly prophylaxis or on demand treatment (n = 15) [26]. Single-dose half-lives were up to 93 h while steady-state geometric mean half-lives were even longer. Mean ABRs were 1.04 (IQR 0.00–4.00) in the 40 IU/kg prophylaxis group (with 67% of patients experiencing no bleeding episodes into target joints), 2.93 (IQR 0.99–6.02) in the 10 IU/kg prophylaxis group, and 15.85 (9.56–26.47) in the on-demand group. No inhibitory antibodies were found during the study period.

A second phase 3 trial included 25 PTPs < 12 years with FIX activity levels ≤ 2% [44]. All patients received once-weekly prophylaxis with 40 IU/kg N9-GP for 50 exposure days. Median ABR was 1.0 (mean ABR for all bleeds 1.44 (95% CI 0.92–2.26)) in the total population. Estimated mean steady-state trough levels were >15% and mean half-life was 70 h in children < 7 years and 76 h in older children. None of the study participants developed an inhibitor. N9-GP was also shown to be safe and effective in a perioperative setting [45].

Monitoring Extended Half-Life Factor VIII and Factor IX Products

For factor activity measurement several methods like one-stage clotting assays (based on activated partial thromboplastin time) and chromogenic methods are used. However, for some of the

products, there are significant differences between results obtained using chromogenic or one-stage assays as well as between results generated using different reagents for the one-stage assay. Briefly, chromogenic assays reveal robust results for monitoring EHL FVIII and FIX concentrates while performance of one-stage assays is dependent on the reagent used [46]. Details concerning performance of particular assays on particular EHL products are beyond the scope of this review. An excellent review paper covering this difficult topic has recently been published by Kitchen and colleagues [46].

Conclusions

EHL FIX products have improved and facilitated prophylactic therapy in hemophilia B remarkably. On the one hand, they allow reduction of injection frequency to once-weekly or even once-biweekly treatments and on the other hand much higher trough levels (FIX > 5% or even > 10%) can be achieved despite reduction of injection frequency [24–26].

With EHL FVIII compounds, advances are not that impressive. Nevertheless, they allow reaching higher trough levels (without reducing injection frequency) in patients who still suffer from breakthrough bleedings despite intensive treatment schedules with conventional FVIII preparations. Furthermore, it is possible with EHL FVIII products to reduce injection frequency in patients who are free of bleedings with conventional FVIII products (e.g. reduction of injection frequency from 3×/week to 2×/week) [47].

In the last 3 years, EHL FIX and FVIII products have already become important instruments to improve hemophilia care in clinical practice. It will be interesting to see how these compounds perform in the upcoming years on a long-term basis and whether EHL FVIII preparations are only an intermediate step in the light of heralded alternative products with much longer half-lives such as emicizumab.

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