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Extended Half-Life (EHL) Coagulation Factors: A New Era in the Management of Haemophilia Patients

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Abbreviations:

ABR	Annualized Bleeding Rates
aPTT	Activated Partial Thromboplastin Time
AUC	Area Under the Curve
BHK	Baby Hamster Kidney
CHO	Chinese Hamster Ovary
CI	Confidence Interval
EHL	Extended Half-Life
EHL-rFIX	Extended Half-Life recombinant Factor IX
Fc	Fragmented crystallisable
FcRn	Neonatal Fragmented crystallisable Receptor
FIX	Factor IX
FVIII	Factor VIII
HEK	Human Embryonic Kidney
HRQoL	Health Related Quality of Life
IgG1	Immunoglobulin G1

ITI	Immune Tolerance Induction
N9-GP	Glycopegylated recombinant Factor IX
OSA	One-Stage clotting Assay
PEG; PEGylation	Polyethylene Glycol
PK	PharmacoKinetic
PKs	Pharmacokinetics
PTPs	Previously Treated Patients
PUPs	Previously Untreated Patients
rFIX	Recombinant Factor IX
rFIXFc protein	Recombinant Factor IX Fragmented crystallisable fusion
rFIXFP	Recombinant Factor IX Albumin fusion protein
rFVIII	Recombinant Factor VII
rFVIIIFc protein	Recombinant Factor VII Fragmented crystallisable fusion
rVIII-SingleChain	Recombinant Single-Chain factor VIII
SHL	Standard Half-Life
SHL-RFIX	Standard Half-Life Recombinant Factor IX
VWF	Von Willebrand Factor

A New Era in the Management of Haemophilia Patients: Replacement Therapy with Extended Half-Life (EHL) Coagulation Factors VIII and IX

Abstract

Despite effective factor replacement and various treatment schedules, there remain several challenges and unmet needs in the prophylactic treatment of haemophilia limiting its adoption and thereby posing an increased risk of spontaneous bleeding. In this regard, extended half-life (EHL) recombinant factor VIII (rFVIII) and factor IX (rFIX) products promise optimal prophylaxis by decreasing the dose frequency, increasing the compliance and improving the quality of life without compromising on safety and efficacy. EHL products might lead to higher trough levels without increasing infusion frequency, or would facilitate ability to maintain trough levels while reducing infusion frequency. This paper aims to provide a comprehensive review on the rationale for developing EHL coagulation factors and their utility in the management of haemophilia, with special emphasis on optimal technique for half-life extension and criteria for defining EHL coagulation factors, as well as indications, efficacy and safety issues of the currently available EHL-rFVIII and EHL-rFIX products. Potential impact of these factors on quality of life, health economics and immune tolerance treatment will be also be discussed alongside the challenges in pharmacokinetic driven prophylaxis and difficulties in monitoring the EHL-products with laboratory assays.

Keywords: *Haemophilia; factor replacement therapy; extended half-life products; laboratory assays; pharmacokinetics; quality of life*

Introduction

Haemophilia A and B are X-linked monogenic inborn coagulation defects that which lead to deficiencies of factor VIII (FVIII) and factor IX (FIX) in approximately 1 of 5,000 and 1 of 30,000 male live births, respectively (1-3).

The disease phenotype is characterized by recurrent spontaneous or traumatic bleeding episodes predominantly involving the weight bearing joints, skeletal muscle, and soft tissues. Intracranial and retroperitoneal haematomas are rare but life-threatening complications of severe haemophilia (1,3). Bleeding phenotype has been defined as "severe", "moderate" and "mild" based on the level of the residual endogenous factor being <1 IU/dL, 1–5 IU/dL and 5–40 IU/dL, respectively (4,5).

Replacement of the missing factor constitutes the mainstay of haemophilia treatment. Factor replacement is given either "on demand" to treat acute bleeding or "prophylactically" to prevent bleeding (2,6). In severe haemophilia, recurrent bleeding, typically in the form of joint bleeds and skeletal muscle haematomas, result in progressive haemophilic arthropathy, muscle contractures which eventually lead to irreversible joint damage, significant disability and decreased quality of life, unless treated with FVIII and FIX (4-8).

Prophylactic regular factor replacement to maintain circulating factor levels > 1 IU/dl (1%) has been recommended as the optimal therapy for people with severe haemophilia, based on the evidence showing that prophylaxis is associated with substantial reduction in bleeding episodes and related complications and consequently with an improvement in the quality of life and life expectancy (9-11).

In the prophylactic setting, people with severe haemophilia A usually require intravenous injections three times a week, while those with severe haemophilia B are usually treated twice weekly, owing to the longer half-life of FIX over FVIII (18–20 h vs. 8–12 h) (2,12).

The relatively short half-life of conventional factor concentrates necessitates frequent intravenous injections to maintain plasma factor levels above the threshold level that protects from bleeding (13,14). However, the requirement for frequent dosing not only creates venous access problems, but also poses an obstacle to patient adherence and proper use and adoption of prophylaxis (15-18). This, in turn may lead to treatment failure resulting in increased disability (19-23). Hence, there is an unmet need for factor concentrates with longer half-lives that would render possible a more successful prophylaxis at less frequent dosing (23-25), and consequently would result in reduced prophylactic treatment burden for patients and caregivers.

Much effort has been devoted to the optimisation of the pharmacokinetics of recombinant factors by molecular modifications to achieve extended half-life (EHL) FVIII and FIX products (3,23,26,27). The new generation EHL factor concentrates are expected to facilitate the implementation of optimal prophylaxis allowing longer treatment intervals without loss of efficacy. Treatment with EHL factors would reduce the burden of frequent intravenous interventions, enable higher adherence to treatment and improve quality of life (4,28-31).

During the past decade numerous techniques have been invented for the development of EHL rFVIII and rFIX molecules, all of which principally exert their effect by decreasing the clearance of the factors. A combination of reduced proteolysis in peripheral blood, decreased renal and hepatic elimination and decreased receptor-mediated endocytosis usually result in prolongation of the half-life of the factor (32). Several novel EHL rFVIII and rFIX products have entered the market or are about to launch following the completion of their phase 3 studies (33-41).

This paper aims to provide a comprehensive review on the rationale for developing EHL coagulation factors and their utility in the management of haemophilia, with special emphasis on optimal technique for half-life extension and criteria for defining EHL coagulation factors,

as well as indications, efficacy and safety issues of the currently available EHL-rFVIII and EHL-rFIX products. Potential impact of these factors on quality of life, health economics and immune tolerance treatment will be also be discussed alongside the challenges in pharmacokinetic driven prophylaxis and difficulties in monitoring the EHL-products with laboratory assays.

Evolution of factor replacement therapy

The current treatment of haemophilia is based upon replacing the missing factor as administered to stop (episodic or on-demand therapy) or to prevent (prophylaxis) bleeding episodes (4,5). The concept of prophylaxis is based on early observations that patients with mild-to-moderate haemophilia (factor levels >1%) bleed less frequently and rarely develop arthropathy (7), and it is now considered as the treatment of choice to increase FVIII to sufficient levels to prevent spontaneous bleeding events and resultant joint damage (17,42). Several studies have unequivocally demonstrated the efficacy of prophylaxis and its superiority to on-demand or episodic therapy in reducing joint bleeding, life-threatening haemorrhages, the risk for hemarthroses and structural joint damage and in improving quality of life (17,43-46). Since 1994 prophylaxis with factor replacement therapy has become standard of care for patients with severe haemophilia and has been shown to prevent arthropathy, if started early in life, or slow the progression of established arthropathy in adults (5,47,48).

EHL coagulation factors: An unmet need

FVIII and FIX are large, complex proteins with relatively short half-life, necessitating frequent dosing to maintain therapeutic levels. EHL coagulation factors are designed to have prolonged half-lives through some structural modifications, such as chemical alterations or fusion of the factor protein to another molecule with a longer half-life. In theory, an extended half-life is expected to result in improved compliance and better prophylactic outcomes by allowing for extended dosing intervals and frequent injections (49).

The optimal method for half-life extension should ensure maintenance of the biological activity of the coagulation factor without increasing the risk for immunogenicity and other safety signals compared with currently available products (49). Definition of a clinically relevant extension of half-life depends on practical considerations, such as the dosing schedule, and the intended clinical application (e.g. on-demand vs. prophylaxis) without compromising on long-term safety (49). The lower clearance rate of EHL factors provides a potential for reducing treatment burden with less frequent injections and equal or improved efficacy without increasing the overall factor consumption (14). This allows greater flexibility for tailoring prophylaxis treatment to the individual needs of the patient, leading to better adherence and consequently the improved standard of care in haemophilia (14,50).

Technologies used for extending half-life of recombinant clotting factors

The strategies to extend the half-life of recombinant clotting factors include a) covalent attachment of FVIII to polyethylene glycol (PEG; PEGylation) to reduce interaction with clearance receptors, b) fusion to the fragmented crystallizable (Fc) portion of immunoglobulin G1 (IgG1) molecule to divert the molecule away from lysosomal degradation to delay its clearance or c) fusion to recombinant albumin, to rescue endocytosed proteins from intracellular degradation pathway and d) single chain technology to increase the stability of the molecule (4,19,51,52).

PEGylation

PEGylation involves covalent attachment of polyethylene glycol (PEG) to FVIII or FIX to enhance the PK and pharmacodynamic properties of the molecules (19) PEGylation increases the circulating half-life for FVIII and FIX potentially by reducing the binding capacity of the PEGylated protein to their clearance receptors and reducing susceptibility of the molecule to proteolytic activity and degradation (19,52). PEGylation of therapeutic molecules has generally been considered to have a low risk of immunogenicity (19). In preclinical trials with BAY 94-9027, it is suggested that this compound was significantly less immunogenic in haemophilia A mice, normal rats and normal rabbits when compared to un-PEGylated rFVIII. However, human data are lacking (53).
PEGylation has been reported to be associated with an approximate half-life extension of 1.5-fold in comparison to the standard half-life (SHL) factors alongside excellent safety and efficacy for the prevention and treatment of bleeding in previously treated adults and children with severe haemophilia A with development of no inhibitory antibodies to FVIII, the PEGylated product or PEG (4,29,33,54-56). Some studies have reported the development of anti-PEG antibodies in patients who received PEGylated therapeutics, it is recommended by FDA that anti-PEG antibody analysis should be done in all subjects who receive experimental PEGylated therapeutics, and evaluation of the potential clinical impact of these antibodies on efficacy and safety (57-59).

Fusion protein technology (Fc fusion and albumin fusion)
Fusion protein technology is the genetic fusion with a protein that has a particularly long half-life, such as immunoglobulins (Fc fusion) or albumin (28,60,61). Albumin and IgG are naturally abundant proteins with a long half-life exceeding 20 days that together account for ~80% of the proteins in plasma, which makes them a subject of common interest in fusion protein technologies (52). The prolonged half-life of albumin and Ig G is due to neonatal Fc receptor (FcRn)-mediated recycling, a naturally occurring recycling pathway that prolongs the half-lives of various biologics by diverting them away from lysosomal degradation, thus delaying clearance and extending the functional plasma half-life (19,49,52).
Recently, albumin fusion technology has been utilised for the half-life extension of rFIX (28).

The mean half-life extension of rFVIII-Fc is about 1.5-fold that of standart half-life FVIII (4,29). Association of albumin fusion technology with a modest increase in half-life of FVIII as compared with the five-fold half-life extension seen with FIX when fused with Fc has been considered to be mainly due to the interaction of FVIII with von Willebrand factor (VWF), as the main regulator of FVIII clearance (54,62).
Results from the pivotal phase III studies A-LONG in adults and adolescents aged> 12 years, Kids A-LONG in children aged ≤ 12 years, and interim evaluation of the recently completed extension study (ASPIRE) have clearly demonstrated the long-term effectiveness and safety of rFVIII-Fc for the treatment and prevention of bleeding in previously treated patients (PTPs) with severe haemophilia A (4,34,63,64).

Single chain technology
Human FVIII circulates as a heterodimeric structure consisting of a heavy (A1-A2-B domains) and light chain (A3-C1-C2 domains) held together by noncovalent interactions, which makes the FVIII molecule relatively unstable, leading to its dissociation under certain conditions and formation of inactivated FVIII chains (4,23,49). A novel recombinant single-chain FVIII (rVIII-SingleChain) has been engineered where the heavy and light chains are covalently linked by a truncated B domain (4,65,66). This single-chain design has been reported to yield a more stable and homogenous product with increased binding affinity for

VWF, and improved pharmacokinetics (PKs) relative to the full-length rFVIII, potentially prolonging the half-life of FVIII (4,23,29). Although, rVIII-SingleChain was well tolerated in the clinical studies without development of inhibitory antibodies, extension of the half-life with this technology is considered to be modest, ranging from 1.1 to 1.4 times the baseline FVIII half-life, with a geometric mean half-life of approximately 14 h (65-67).

Currently available EHL-rFIX and EHL-rFVIII products

Three EHL-rFIX products have completed phase 3 clinical studies and licensed in adolescent and adult patients (26,41), including rFIXFc (Alprolix, Sobi, Stockholm, Sweden; Bioverativ, a Sanofi company, Waltham, MA, USA) (37), nonacog beta pegol (N9-GP, Novo Nordisk A/S, Bagsværd, Denmark) (39) and rFIX-FP (Idelvion, CSL Berhing, King of Prussia, PA, USA) (38) (Table 1).

There are four EHL-rFVIII products that have completed phase 3 clinical studies. rFVIIIFc (Elocta, Sobi, Stockholm, Sweden; Eloctate, Bioverativ, Waltham, MA, USA) (34), and octocog alfa pegol (Adynovate, BAX855, Baxalta, Vienna, Austria) (33) are licensed in some countries. Turoctocog alfa pegol, (N8-GP, Novo Nordisk A/S, Bagsværd, Denmark) (35) has completed a phase 3 study and phase 2/3 data are published for BAY 94-9027(Jivi, Bayer Healthcare AG, Leverkusen, Germany) (56). Finally, a B-domain–truncated single-chain rFVIII concentrate, ScrFVIII (Afstyla), has recently been licensed by CSL Behring in some countries (40). However, this product displays a modest extension in half-life and is not regarded as an extended half-life concentrate (Table 1).

Considering mechanisms of half-life extension in rFIX concentrates, rFIX-Fc (Alprolix) fuses the Fc immunoglobulin region with FIX, while rFIX-FP (Idelvion) combines FIX with albumin, and N9-GP (Rebinyn/Refixia) is a pegylated version of FIX. As with EHL-rFIX concentrates, EHL-rFVIII concentrates are also based on Fc fusion (rFVIIIFc, Elocta/Eloctate) or pegylation (N8-GP; BAX 855 and BAY 94-9027). BAY 94-9027 and N8-GP are B-domain-deleted (BDD) rFVIII, whereas BAX855 is a full-length rFVIII (6,41,56,68) (Table 1).

Table 1. Currently available EHL rFVIII and rFIX concentrates (41)

	Generic name	Company	Mechanism	Half-life (h)	Current status	Reference
Factor VIII – extended half-life concentrates						
Elocta/Eloctate (rFVIII-Fc)	Efraloctocog alfa	Bioverativ/Sobi	Fc (IgG1) fusion to B-domain deleted FVIII	19.0	Licensed	34
Adynovate (BAX855)	Octocog alfa pegol	Shire	Pegylated to full length FVIII, porcine sequence	14.3	Licensed	33
N8-GP	Turoctocog alfa pegol	Novo Nordisk	Pegylated (40 kDa) B-domain truncated FVIII	19.0	In Phase 3 trials	35
Jivi (BAY94-9027)	Damoctocog alfa pegol	Bayer	Pegylated (60 kDa) B-domain deleted FVIII	18.7	Licensed	36
Factor IX – extended half-life concentrates						
Alprolix	rFIX-Fc	Bioverativ/Sobi	Fc fusion to FIX	82.1	Licensed	37
Idelvion	rFIX-FP	CSL Behring	Albumin fusion to FIX	102	Licensed	38
Rebinyn/Refixia (N9-GP)	Nonacog beta pegol	Novo Nordisk	Pegylation of FIX	96.3	Licensed	39

The main characteristics of EHL concentrates are provided in Table 2. For all three EHL-rFIX, an unmodified native rFIX protein was used, while an increase in the extension of rFIX half-life (3.8-fold, ranged from 2.4 to 4.8-fold) and extension in the dosing frequency for prophylaxis (ranging from 7 to 14 days) were evident when compared to the standard half-life rFIX (SHL-rFIX) (26). Treatment of bleeding episodes as well as prophylaxis with all three EHL-rFIX products has been reported to be successful providing evidence for high overall haemostatic activity. Bleeding episodes could effectively be treated with 1–2 injections. A consistent decrease in clearance and increased area under the curve (AUC) as well as an increased incremental recovery were noted in all three EHL-rFIX for the same dose of 50 IU/kg of the EHL-rFIX in comparison to SHL-rFIX, leading to substantial and meaningful prolongations of half-life and justifying a once weekly dosing regimen. Overall, the safety profile of all three EHL-rFIX products were satisfactory in the adolescent and adult setting with no sign of inhibitor development or drug-related serious adverse events (Table 2).

Half-life extension of EHL-rFVIII products is in the range of 1.4–1.6 fold and the annualized bleeding rates (ABR) were below 4 bleeds per year with a range from 1.3 to 3.6 bleeds. In patients who experienced bleeds, these were successfully treated with the EHL rFVIII; more than 96% of bleeds resolved with one or two injections of the products. Haemostatic efficacy was rated good or excellent in more than 90% of the bleeding episodes. No inhibitor development has been reported in clinical trials with the EHL-rFVIII products (Table 2).

Data on the use of EHL products in the paediatric age group (64,69-71) revealed low median ABRs ranging from 1 to 3 bleeds with no major differences between the products and no inhibitor development (26). Modest increase in the half-life of the FVIII products achieved through extension techniques could only reduce the treatment frequency to twice weekly.

For rVIII-SingleChain PK profile, safety and efficacy are under investigation in the frame of a unique clinical trial program designed as a large interconnected series of Phase I/III studies referred to as the AFFINITY clinical trial program (29,66,72). Preliminary data from this program showed an excellent/good haemostatic efficacy in both, prophylactic and episodic treatment with a good safety profile. No inhibitor development has been reported with rVIII-SingleChain so far (72). PK analysis showed a favourable PK profile for rVIII-SingleChain over full-length rFVIII, though the half-life was relatively shorter and the clearance relatively higher in the paediatric group (73).

Table 2. Characteristics of EHL rFIX and EHL-FVIII products

	EHL-rFIX products					
	rFIXFc (Alprolix) (37)		**rFIX-FP (Idelvion) (39)**		**N9-GP (Rebinyn/Refixia) (38)**	
General properties						
rFIX protein	non-modified		non-modified		non-modified	
Half-life extension moiety	Fc portion of Ig		recombinant human albumin		40kDa polyethylene glycol moiety	
Linking method	fusion of Fc to rFIX		fusion of recombinant albumin to rFIX		site-directed glycopegylation of rFIX	
Cell line	HEK cells		CHO cells		CHO cells	
Mechanism of half-life extension	FcRn recycling		FcRn recycling		decreased renal filtration, proteolytic degradation and receptor-mediated clearance of protein	
Half-life properties						
Mean half-life for EHL product	82.1		102		96.25	
Mean half-life for standard rFIX	33.8		24.2		19.3	
EHL-rFIX to SHL-rFIX ratio	2.4-fold		4.2 fold		4.8-fold	
Pharmacokinetic/dynamic properties	**rFIXFc (Alprolix)**		**rFIX-FP (Idelvion)**		**N9-GP(Rebinyn/Refixia)**	
Dose used, IU/ kg	50		50		40	
Area under the curve (AUC), h-IU/dL	3664		7176		14 130	
Clearance, mL/ kg	0.74		0.77		0.42	
Incremental recovery, IU/dL / IU/ kg	0.92		1.27		2	
Efficacy	**rFIXFc (Alprolix)**		**rFIX-FP (Idelvion)**		**N9-GP (Rebinyn/Refixia)**	
Dose, IU/kg	50	100	40	75	10	40
Dosing frequency, days	Every 7 days	Every 10/14 days	Every 7 days	Every 14 days	Every 7 days	Every 7 days
Number of participants	61	26	40	21	30	29
Annualized bleeding rate, median (IQR)	3.0 (1.0, 4.4)	1.4 (0.0, 3.4)	0.0 (0.0, 1.87)	1.08 (0.0, 2.7)	2.93 (0.9, 6.0)	1.0 (0.0, 4.0)
No of injections required for bleed resolution	1–2		1–2		1–2	
Overall hemostatic efficacy, %	97.2		96.7		97.1	
Safety profile	**rFIXFc (Alprolix)**		**rFIX-FP (Idelvion)**		**N9-GP (Rebinyn/Refixia)**	
No of patients with inhibitors	0		0		0	
No of patients with non-inhibitor antibodies	3		0		3	
No of deaths, thromboembolism	0		0		0	
No of drug-related serious adverse events	0		0		0	
No of drug-unrelated serious adverse events	11		3		4	

	EHL-FVIII products			
	BAY94-9027(Jivi) (35)	**BAX855 (Adynovate)(36)**	**rFVIIIFc (Elocta/Eloctate)(34)**	**N8-GP (33)**
General properties				

rFIX/rFVIII protein	B-domain deleted rFVIII	Full-length rFVIII	B-domain deleted rFVIII	B-domain deleted rFVIII
Half-life extension moiety	60 kDa PEG molecule	2 x 20 kDa PEG molecule	Fc portion of Ig	40 kDa PEG molecule
Linking method				
Cell line	BHK	CHO	HEK	CHO
Mechanism of half-life extension	decreased renal filtration, proteolytic degradation and receptor-mediated clearance	decreased renal filtration, proteolytic degradation and receptor-mediated clearance	neonatal Fc receptor recycling	decreased renal filtration, proteolytic degradation and receptor-mediated clearance
Half-life properties				
Mean half-life for EHL product	18.7	14.3	19.0	19.0
Min-max half -life	13.7–28.1	14.3–16.0	17.1–21.1	11.6–27.3
Fold increase in half-life	1.4	1.4	1.5	1.6
Efficacy				
Bleeds resolved with 1 injection, %	-	85.4	87.3	84
Bleeds resolved with 1 or 2 injections, %	-	95.9	97.8	96
ABR on prophylaxis, median	2.88 (1 x week)	1.9 (2 x week)	1.6 (every 3-5 day)(ref. 34)	1.3 (q4 days)
Overall hemostatic efficacy, %	>90	>90	>90	>90
Safety				
No of patients with non-inhibitor antibodies	-	-	-	-

Pharmacokinetics of EHL rFVIII and rFIX products in children

	Half-life	Dosing	Reference
rFVIII Fc	14.9 (in 6–12 year olds) 12.7 (<6 year olds)	Twice weekly with 25 IU/kg on day 1 and 50 IU/kg on day 4	65
rFIX Fc	70.3 h (6–12 year olds) 66.5 (<6 year olds)	50–60 IU/kg once weekly	70
rFIX-FP	91 h	50 IU/ kg once weekly	71
N9-GP	76.3 h (6–12 year olds) 69.6 (<6 year olds)	40 IU/kg once weekly	69

EHL-rFIX: extended half-life recombinant factor IX; rFIXFc: recombinant factor IX Fc fusion protein; rFIXFP: recombinant factor IX Albumin fusion protein; N9-GP: glycopegylated rFIX; FcRn: neonatal Fc receptor; AUC: area under the curve, EHL-rFIX: extended half-life rFIX, SHL-RFIX: standard half-life rFIX, BHK: baby hamster kidney; CHO: Chinese hamster ovary; HEK: human embryonic kidney, PEG: polyethylene glycol

Criteria for classifying a replacement factor as an EHL product

The advent of EHL recombinant factors, bioengineered to have a longer half-life than standard recombinant proteins, has been an important evolution in concentrate manufacturing providing an important expansion of treatment options to be considered as part of individualized haemophilia care (41,74,75). However, each new treatment option that becomes available gives rise to the concerns regarding the optimal utility to provide the best possible outcome for each patient (52).

Ideally, EHL recombinant factors should allow reduced dosing frequency with retention of haemostatic efficacy compared to SHL recombinant factors for the majority of patients (76). However, the current literature does not provide clarity regarding the clear distinctions between EHL and standard products along with no definition criteria established to classify a recombinant factor as an EHL, and lack of a critical assessment to determine which EHL products fulfil the requirements (76).

Given the tight non-covalent association of FVIII with VWF in the circulation which imposes a biological limit on the extension of the half-life of FVIII beyond that of VWF, the EHL rFVIII products have not had an equally substantial improvement in half-life as observed with EHL rFIX products (76,77). EHL rFIX products demonstrated a 3-5-fold increase in half-life compared to standard FIX concentrates, providing a clear threshold for differentiating the extended half-life products from the standard ones (76,78).

However, this is not always the case for FVIII products. In a modelling study designed to identify the minimum half-life extension ratio required for a reduction in dosing frequency while maintaining the proportion of patients with plasma rFVIII levels > 1 IU/dL with no increase in the total weekly dose, authors noted that a meaningful reduction in the burden of infusions for an EHL rFVIII product (relative to a standard rFVIII) is possible when the half-life extension ratio is 1.3 or greater (79). In addition, it has been suggested that both AUC ratio and half-life ratio should be used to provide sufficient PK evidence for a solid definition of EHL (76). Accordingly, to be attributed as "EHL product" a FVIII concentrate should fulfil all of the following 3 criteria: 1) it should be designed and engineered with a relevant technology used to extend circulating biological half-life, 2) difference from the standard rFVIII comparator should be demonstrated for the majority of patients according to the proposed "bio-difference" criteria based on the lower limit of the 90% CI for the AUC ratio being above the FDA/EMA cut-off for bioequivalence (1.25 or 125%) and 3) a half-life ratio of 1.3 or higher, based on modelling, should be available (76,79).

BAX 855 and rFVIIIFc have been reported to clearly comply with all 3 of these criteria, while rFVIII-SingleChain failed to fulfil the criteria since it cannot be fully differentiated from the standard rFVIII (Advate®), based on the 90% CIs for AUC ratio extending below 1.25 and a half-life extension ratio of 1.09 when compared to Advate®. This suggests that rFVIII-SingleChain may behave like a standard rFVIII in some patients despite its modified PK characteristics (76). Although there are some limitations imposed by the different study designs and reporting, current evidence suggests that both BAY 94-9027 and N8-GP fulfil criteria for EHL rFVIII and these agents should also be classified as EHL rFVIII products (76).

Definition and classification is always of help for a better understanding of the potential benefits and limitations of recombinant factor products. However, one should never forget that these cannot substitute for careful clinical monitoring of patients, including measurement of rFVIII levels and individual PK profiles.

Indications and utility of EHL: Switching from standard half-life factors to EHL factors

The published phase I-III studies on prophylaxis with EHL rFVIII and rFIX products revealed improved PK properties of the bioengineered molecules with a half-life extension ranging 1.2- to 1.5-fold for FVIII and 3- to 5-fold for FIX (19). EHL products were shown to be well tolerated with no inhibitor development in the PTP population. They were efficacious in the treatment and prevention of bleeding episodes with the potential to reduce the burden of infusions and/or achieving higher trough levels (19,74,76).

Market availability of effective EHL products with a potential of reducing infusion frequency will inevitably induce a transition from SHL to EHL factor concentrates, in both the episodic treatment and prophylaxis setting. Extension of half-life might lead to higher trough levels without increasing infusion frequency, or would facilitate ability to maintain trough levels while reducing infusion frequency. Either of these strategies could be implemented to improve outcomes, depending on the characteristics of the patient (74,75). In a study using dosing simulations to investigate potential clinical outcomes via different prophylactic regimens with rFVIIIFc and rFVIII, authors suggested that patients with different needs might benefit in different ways from transitioning from rFVIII to rFVIIIFc (14). A high correlation in PK data between rFVIIIFc and rFVIII was also noted with a one-third lower average clearance for rFVIIIFc, which could be useful for adjusting doses in case of a transition between the two products (14).

Accordingly, *"standardised"* (fixed dose and interval; once weekly for FIX and twice weekly for FVIII), *"PK-driven"* (dosed to a target trough, fixed interval), *"phenotype-driven"* (variable dose and interval according to bleeding pattern and activity), and *"convenience-driven"* (higher dose, longer interval) strategies have been used for the prophylaxis regimens in pivotal clinical trial programs (19).

There might be a concern regarding inhibitor development when switching between different FVIII concentrates as product type is one of treatment-related factors on inhibitor development (77). Recent real-world data on EHL factor concentrates are in support of the data obtained from previous clinical studies with these products in PTPs stating that no inhibitor formation was observed in patients who switched from conventional factor VIII or IX replacement to treatment with EHL factor VIII or IX (78-80). In non-adherent patients switching to a standardised prophylaxis regimen with EHL factors (once or twice weekly) has been associated with a successful treatment outcome leading to trough levels sufficient to suppress target joint bleeding (19). Patients who were bleeding under conventional rFVIII treatment have been shown to benefit both from improved bleed control and reduced injection frequency when switched to rFVIIIFc prophylaxis at similar prophylactic factor consumption (14,84). Thus, the same prophylactic total weekly dose might be given initially, in divided daily doses, twice weekly instead of 3 times a week when switching from SHL FVIII to rFVIIIFc. Thereafter, the dose and dosing interval can be adjusted depending on the patient's clinical needs (14).

Accordingly, data from ALONG trial showed that 30% of Haemophilia A patients in the individualized prophylaxis arm achieved 5-day dosing intervals in the last 3 months of the study (34). In ASPIRE, the phase 3b extension study, interim data revealed that further prolongation of dosing intervals to 7-day intervals was possible in 2 of the 33 patients who were on twice weekly dosing and 10 of 37 who were on every 5-day dosing in the ALONG study (59). Overall, median ABRs were lower with rFVIIIFc prophylaxis (individualized prophylaxis: 0.66, weekly prophylaxis: 2.03; modified prophylaxis: 1.97) as compared with on-demand treatment (18.36) (63). A 30% lower total weekly dose of rFVIIIFc has been shown to be likely to give the same FVIII exposure considering that rFVIIIFc has a 30% lower clearance when compared to rFVIII (14). Based on this, one can conclude that patients who are well controlled on prophylactic treatment with a conventional FVIII product might maintain the same level of bleed control while benefiting from reduced injection frequency and decreased prophylactic factor consumption when switched to EHL-rFVIII. However, this has to be further investigated in clinical trials.

While the primary focus has been the potential use of EHL factor concentrates in patients with severe haemophilia, utility of EHL products for the management of bleeding in patients with mild and moderate deficiencies has also been considered (52).
Further support with real-life data on the potential advantages of EHL factor concentrates may enable understanding individual patient characteristics and treatment needs and the differences in behaviour of conventional FVIII products as compared to EHL products. This may help guide clinicians when switching haemophilia A patients from conventional FVIII to EHL products.
Nonetheless, it should be noted that commenting on the comparative efficacy of new long-acting therapies is not possible in the lack of head-to-head studies. Furthermore, it is difficult to compare different EHL products with the current clinical data since the published studies greatly vary with regard to study populations, study designs and protocols and evaluate outcome using different end-points such as ABR, inhibitor development, number of breakthrough bleeds, dosing intervals, etc.

Current evidence on the impact of quality of life and health economics

Prophylaxis is considered the best practice for countries in which it is economically affordable (41,85,86), and is associated with a better health related quality of life (HRQoL) as compared to episodic treatment (87). Furthermore, it leads to a decrease in bleeding-related hospitalization, shortens length of hospital stay, and thus reduces resource utilization (85,88).
EHL factors offer prolonged protection from bleeding episodes, while reducing the frequency of infusions and allowing trough activity levels to remain above key thresholds for longer periods relative to conventional factor products (19). Thus, longer half-lives and reduced clearance with EHL factors are suggested to lead to reduced factor consumption, while maintaining or improving protection from bleeding, resulting in considerable reductions in haemophilia-related complications and their associated cost burden (19).
In two phase III studies done with rFVIIIFc and rFIXFc HRQoL was assessed in adults with severe haemophilia A and B, respectively, who received prophylactic or episodic factor replacement regimens (34,37). The post hoc analyses of these studies revealed that prophylaxis with rFVIIIFc or rFIXFc was associated with significant improvements in HRQoL (particularly in 'Physical Health' and 'Sports and Leisure' domains) over time. This has also been noted in patients who had been receiving prophylaxis with SHL-rFVIII/FIX and were switched to EHL product rFVIIIFc and rFIXFc. Thus, EHL factor concentrates seem to further improve HRQoL of haemophilia patients (87).
In an analysis of the potential budget implications of introducing rFVIIIFc to a private payer formulary in the US, rFVIIIFc is anticipated to have a budget impact of 1.4% across 2 years for a private payer population of 1,000,000 and to reduce ABRs by approximately 3.1 bleeds per individual with haemophilia A (85). The total population budget was predicted to decrease for episodic treatment with the introduction of rFVIIIFc, based on consideration of patients switching to prophylaxis with rFVIIIFc, and a reduction in per-patient costs for rFVIIIFc relative to other products on the basis of lower factor consumption observed in the ASPIRE trial (63,85).
For prophylaxis, the cost per bleed avoided after the introduction of rFVIIIFc was estimated to be $1974 in year 1 and $1808 in year 2, while the small decrease in cost per bleed avoided over time was considered to be associated with likelihood of patients uncontrolled on a fixed prophylaxis regimen to switch to an individualized regimen in year 2, resulting in a more efficient use of factor therapy (85).
In the scenario without rFVIIIFc, the annual cost of episodic therapy was estimated to be $2,044,868 and the estimated number of bleeding episodes among patients receiving episodic therapy was 388, which equates to each bleeding episode costing approximately $5,270 ($2,044,868/388 bleeds). The cost per bleed avoided with rFVIIIFc on the market was

approximately $1,891 indicating that prophylaxis with rFVIIIFc provides good value for money in the prevention of joint bleeds and related comorbidities (85).
The introduction of rFVIIIFc to a private payer formulary is anticipated to have a minimal budget impact while reducing the ABR, alongside a potential of reduced dosing schedule required for rFVIIIFc and reduction in total factor consumption, facilitating adherence to prophylaxis regimens, with a likely positive impact on patient quality of life and economic burden (85).

Role of EHL factors in immune tolerance treatment

Occurring in up to 30% of patients with severe haemophilia A, the development of alloantibodies (inhibitors) directed against FVIII is the most serious complication of replacement therapy (2), leading to treatment failure, preventing patients from receiving long-term prophylaxis and exposing them to an increase risk of mortality, morbidity, and disability (2,6,89).
The current management of patients with inhibitor development is to treat acute bleeding with agents that bypass the need for FVIII or FIX, i.e. activated prothrombin complex concentrate, or activated recombinant factor FVII, and using immune tolerance induction (ITI) in order to eradicate the inhibitory antibodies (89). Several protocols of ITI have been published worldwide with approximately a 70% overall success rate (89,90).
While several meta-analyses failed to demonstrate a significant difference of inhibitor occurrence in patients treated with recombinant FVIII compared to plasma-derived products, a randomized prospective study revealed that the use of recombinant products in previously untreated patients (PUPs) was associated with a 1.8 greater risk of inhibitor development compared to plasma-derived products (91). There is no published study to date presenting definitive results with novel EHL concentrates in PUPs, while all the studies in PTPs showed an excellent safety profile with substantially no inhibitor occurrence after switching to the novel products (6). Nonetheless, at the immunogenicity level, so far, available data from clinical trials that were conducted in PTPs suggest that all forms of EHL factors are safe, as no inhibitors have been reported in these patients (34,64,89).
Potential role for the EHL factor for the induction of immune tolerance after the development of inhibitors has also been suggested (19,52). A series of case reports regarding use of EHL rFVIII in ITI, described the successful treatment of children with severe haemophilia A and high-titer inhibitor using different doses of rFVIIIFc in ITI ranging from 50 to 200 IU/kg per dose (92,93). Hence, with no reports of any EHL factors causing inhibitor formation in the initial clinical trials and awaiting more clinical data or randomized trials in PUPs and in ITI, the rationale of safely using rFVIIIFc is well supported by case reports and strong laboratory data (52,89). Available evidence encourage the consideration of the use of rFVIIIFc to eradicate inhibitors, particularly in refractory patients and those with a high-risk profile (i.e. those with a family history of failure of ITI with standard factors or history of a high-peak inhibitor) (52,89).

Challenges in pharmacokinetic- guided prophylaxis with EHL products

Although EHL concentrates are increasingly used in clinical practice, implementing individualized prophylaxis regimens with dosing based on desired trough levels using those EHL products represent a challenge for an optimal management of the disorder (6,13).
Prophylactic dosing in severe haemophilia is generally tailored according to individual needs of the patients. Tailoring of treatment has been guided by either clinical bleeding phenotype or individual pharmacokinetics of a particular factor concentrate in a patient (19).
PK-tailored prophylaxis was shown to have superior haemostatic efficacy over on-demand treatment, along with decreased factor consumption (17). PK-tailored dosing, explored in several of the phase 3 clinical trials with EHL factors, was associated with good efficacy in bleed control in

these prospective studies (19). Hence, all licensed EHL products recommend tailoring the dose to the individual patient's pharmacokinetic response, since standardized dosing may result in patients being undertreated if factor clearance is higher than expected (41).

The individualized PK-based dosing is considered an alternative option for prophylaxis with some standard FVIII replacement therapies to determine the dose and schedule needed to maintain a predetermined factor trough level (94,95). However given the burden and cost of frequent blood sampling required for personalized PK assessment and likelihood of lack of ready access to the expertise required for such evaluations, the current prescribing information for the available EHL–FVIII products does not follow individualized PK-based dosing (94), while, the recommended fixed dose is based on individualized PK-guided dosing in some clinical trials (94). Unlike haemophilia A, PK-guided prophylaxis has a limited value in most adult patients with haemophilia B on standard FIX products (96,97) and PK-guided dosing strategies for EHL–rFIX products is considered likely to be challenging due to the interindividual variability and complexity of FIX PK, and uncertainty regarding the optimal sampling time that best accounts for a prolonged half-life (98). Accordingly, population-based PK estimation with reduced sampling is considered a more convenient and less costly PK-based estimate of factor requirements than an individualized approach in both haemophilia A and haemophilia B (94,98).

In addition, there are several challenges with PK-guided prophylaxis when EHL factor concentrates are used (99). In contrast to available conventional FVIII products which present with approximately the same PK characteristics enabling similar treatment outcome when used interchangeably, EHL products demonstrate unique pharmacokinetics resulting in different dose and dosing interval requirements and consequently variable treatment outcome (14). Long-term outcomes are also lacking for using a low ABR as a surrogate for the likelihood of either preserving healthy joints with primary prophylaxis or delaying the progression of joint disease in secondary prophylaxis (19,100).

Given the unpredictable impact of the bioengineering approach within individual patients, increased knowledge on the PK parameters of new anti-haemophilic molecules with prolonged half-lives is considered to improve tailoring patients' prophylactic protocols according to patient individual needs and PK characteristics and thus to offer new possibilities for improving prophylaxis effectiveness using individualized target trough levels for different patient profiles (17,19). However, population PK models with the EHL factors are not yet available for routine clinical use to help guide PK tailoring (19).

Challenges in monitoring treatment via laboratory assays

Monitoring treatment through laboratory assays is an important part of ensuring patient safety. Commonly used laboratory methods for measuring FVIII or FIX activity may not be optimal for some modified rFVIII or rFIX products given that modifications applied to the molecules to extend half-lives such as PEGylation, and fusion to albumin or immunoglobulin has introduced variations in their activity measurement with routine factor assays (68,101).

While measurement of recombinant coagulation factor concentrates has always been complicated by discordance between the measurements carried out with different types of assays (68), the modifications applied to extend the half-life of rFVIII and of rFIX have created novel interactions with the reagents of the coagulation assays (68) and created challenges for laboratories that traditionally use one-stage aPTT factor assays to assess therapy (101). Although it differs between different EHL products to which extent the accuracy of laboratory assays are affected (i.e. how well assay monitoring works) (68), the heterogeneity in the assay monitoring is considered to be associated with clinically significant over- or underestimation of plasma concentration with the potential to adversely affect patient management and an unnecessary search for inhibitor antibodies (68,101).

Besides the modification of the rFVIII and rFIX molecules specifically aimed at prolonging the half-life, several characteristics of the unmodified molecules themselves, as well as of the assays, along with use of different calibration methods contribute to the discrepancies in the measured recoveries (68,102).

Chromogenic assays show less variability than one-stage aPTT-based clotting assays in the measurement of FVIII activity levels, possibly due to a smaller number of available assay kits and reagents (103,104). Characterization of potency assays for various modified rFVIII products has revealed that chromogenic assays are robust across different kits (23,101,103,105).

Hence, chromogenic substrate assay is frequently considered the assay of choice for monitoring patients treated with several EHL rFVIII or rFIX concentrates. However, several challenges are associated with the implementation of chromogenic assays in routine clinical laboratory practice including a perception of higher associated costs compared to the one-stage clotting assay (OSA) and greater technical complexity (103) alongside its availability only in a small number of laboratories and a higher inter-laboratory variability when compared with the OSA, especially at low FVIII concentrations (68,106).

Concordance between assays used in laboratories and by pharmaceutical companies to measure potency of the product, effective communication between the laboratories and the clinicians as well as conveyance of relevant information by companies for correct monitoring of their products to both local laboratories and clinicians are essential for a proper monitoring (68,101). Further laboratory and clinical studies are required for the utility and optimization of the laboratory assays with regard to correctly measuring the activity levels of novel EHL rFVIII and rFIX concentrates (68).

Conclusion

EHL factor concentrates have been shown to be well-tolerated and efficacious in the treatment and prevention of bleeding episodes with a potential to reduce the burden of infusions and/or achieve higher trough levels facilitating adherence to prophylactic regimens (19,76,85). Moreover, significant improvements in HRQoL have been shown with EHL factors in a large proportion of subjects including those who have been on prophylaxis with SHL products. EHL products seem to have filled this gap by increasing adherence and further improving the HRQoL of haemophilia patients (87). In addition to that, usage of EHL factors in PTPs has not been associated with increased inhibitor formation. Results of the clinical studies in PUPs and in the setting of ITI are eagerly awaited. However, available data with rFVIIIFc encourage the consideration of the use of EHL products in ITI protocols to eradicate inhibitors, particularly in refractory patients and those with a high-risk profile (52,89).

Given the evolutions in the treatment of haemophilia in recent years with the advent of multiple non-replacement treatment options entering the market, clinicians and patients now face the prospect of having a variety of choices for individualising the treatment according to their needs (41). EHL-rFIX and rFVIII products have already become important alternatives in improving haemophilia care in clinical practice, while their performance on a long-term basis remains unknown. Furthermore, the potential impact of different mechanisms of half-life prolongation on long-term safety (i.e., fusion technology versus PEGylation) is yet to be clarified, alongside the issues of immunogenicity in PUPs and cost (29). EHL- factor concentrates will very soon be challenged by alternative products with even longer half-lives and subcutaneous administration or potentially gene therapy. Exciting developments are about to occur in the near future of haemophilia treatment and we have to wait until all the battle lines are drawn and new options fall into place before discussing which one is most optimal.

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