Recent advances in haemophilia treatment

D. van den Bossche, MSc[1], M. Jacquemin, MD, PhD[1-4], K. Peerlinck, MD, PhD[2-4]

SUMMARY

In the past few years, several new treatment options for haemophilia A and B have emerged. Formerly, replacement therapy comprised plasma-derived and recombinant factor VIII and IX concentrates containing human- and animal-derived components associated with a potential risk of contamination with infectious agents. Further optimisation of the manufacturing procedures virtually eliminated these hazards. Nowadays, the major drawbacks of the standard plasma-derived and recombinant factor VIII and IX products are their relatively short half-life. To overcome these limitations, different therapeutic approaches were developed. Novel extended half-life rFVIII and rFIX concentrates allow a reduction of the injection frequency and improve the efficacy of therapy and the quality of life of haemophilia patients. Besides the prophylactic treatment options, important progress has been made in gene therapy. Currently, the major complication of the treatment with FVIII or FIX concentrates is the development of inhibitor antibodies. In these cases, bypassing agents allow treating or preventing bleedings. However, the currently available bypassing agents have a short half-life, which limit their use for prophylactic treatment. Accordingly, several new therapies are now being developed to treat patients with inhibitors, including rFVIIa with extended half-life, recombinant porcine FVIII and bispecific antibodies bridging FVIII and FIX.

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INTRODUCTION

Haemophilia A and B are congenital, X-linked haemorrhagic diseases caused by genetic defects in the coagulation factor VIII (FVIII) and IX (FIX) gene, respectively. Both bleeding disorders are characterised by dysfunctional or deficient FVIII or FIX proteins. Depending on the severity of the disease and the corresponding residual coagulation factor activity, spontaneous or traumatic haemorrhages occur mainly in joints, soft tissues and muscles. Recurrent intra-articular bleedings can result in chronic arthropathy with joint deformation and loss of function.

Replacement therapy of the deficient coagulation factor is the standard of care in haemophilia patients.[1] The first recombinant FVIII (rFVIII) concentrates, introduced in the 1990s, contained human serum albumin and animal products in the production process, which presented a potential risk of contamination with infectious agents. Modifications in the manufacturing procedures allowed the progressive elimination of human- and animal-derived material. Several rFVIII concentrates fully devoid of human- and animal-derived components are now distributed in Belgium. For example, Advate (Baxalta/Shire) and Kovaltry (Bayer) are full length rFVIII concentrates.[2] ReFacto AF (Pfizer) and Nuwiq (Octapharma), the first rFVIII produced in a human cell line, are B-domain deleted rFVIII concentrates.[3,4] Afstyla (CSL Behring), approved in 2017 by the European Medicines Agency (EMA), is a single-chain rFVIII concentrate, with a truncated B-domain and a covalent link between the heavy and light chains of FVIII.[5] Furthermore, one recombinant factor IX (rFIX) concentrate, BeneFIX (Pfizer) is available.[6] In addition, two plasma-derived FVIII concentrates are still accessible, Octanate (Octapharma) and Factane (LFB).[7]

[1]Department of Laboratory Medicine, University Hospitals of Leuven, Leuven, Belgium, [2]Center for Molecular and Vascular Biology, University of Leuven, Leuven, Belgium, [3]Department of Vascular Medicine and Haemostasis, University Hospitals Leuven, Leuven, Belgium, [4]National Coordinating Haemophilia Centre, University Hospitals Leuven, Leuven, Belgium.

Please send all correspondence to: D. van den Bossche, MSc, Department of Laboratory Medicine, University Hospitals of Leuven, Herestraat 49, 3000 Leuven, Belgium, tel: 0032 16347034, email: dorien.vandenbossche@uzleuven.be.

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TABLE 1.

EHL rFVIII/ rFIX products		Tradename	Marketing authorisation status	Reported half-life (hour)†‡	Reference
PEGylated proteins					
FVIII	N8-GP BAY 94-9027 BAX 855	- - Adynovate	Clinical trial Phase III Clinical trial Phase III Approved by FDA	23.1 (Range: 11.57-27.35)* 18.5 (Range: 15.1-23.4)* 14.3 (95%-CI: 6.7-21.9)§	13 11 14
FIX	N9-GP	Refixia	Approved by EMA	85.1 (95%-CI: 47.7-122.5)§	16
Fc-fusion proteins					
FVIII	rFVIII-Fc	Elocta (EU) Eloctate (USA)	Approved by EMA/FDA	18.8 (95%-CI: 14.3-24.5)§	18
FIX	rFIX-Fc	Alprolix	Approved by EMA/FDA	82.1 (95%-CI: 71.4 - 94.5)§	22
Albumin-fusion proteins					
FIX	rIX-FP	Idelvion	Approved by EMA/FDA	91.6 (95%-CI: 50.1 - 133.1)§	24

† To compare with reported $t_{1/2}$ for standard rFVIII (11-16 hours) and reported $t_{1/2}$ for plasma-derived FVIII (11-15 hours) [29];
‡ To compare with reported $t_{1/2}$ for standard rFIX (18-24 hours) and reported $t_{1/2}$ for plasma-derived FIX (29-43 hours)[30];
* Pharmacokinetic study was performed with measurement of FVIII activity by a chromogenic substrate assay;
§ Pharmacokinetic study was performed with measurement of FVIII/FIX activity by a one-stage assay;
CSA: chromogenic substrate assay; EMA: European Medicines Agency; FDA: US Food and Drug Administration; OSA: one-stage assay; PEG: polyethylene glycol; rFVIII: recombinant factor VIII; rFIX: recombinant factor IX.

Current guidelines recommend prophylactic treatment in the management of severe haemophilia patients (FVIII <1% of normal). Prophylaxis is intended to prevent haemorrhages, joint destruction and ultimately to preserve a normal musculoskeletal function. However, the short half-lives of FVIII and FIX necessitate frequent administration of the standard replacement products.[1,8] To overcome this limitation, rFVIII and rFIX concentrates with extended half-lives (EHL) and alternative therapies such as gene therapy have been developed.

Despite the progress made in the production of FVIII and FIX concentrates, a major treatment complication with these products remains the development of inhibitor antibodies. Bleeding episodes in patients with an inhibitor to FVIII or to FIX can be treated with bypassing agents such as Feiba (Baxalta/Shire) and NovoSeven (Novo Nordisk).[9,10] However, these agents have a short half-life, which limits their use for prophylactic treatment. Accordingly, several new therapies are now being developed to treat patients with an inhibitor, including long-acting activated rFVII (FVIIa), recombinant porcine FVIII and bispecific antibodies bridging FVIII and FIX.

LONG-ACTING RFVIII AND RFIX

To enhance the *in vivo* survival of rFVIII and rFIX, the coagulation factors were coupled to large multimers of polyethylene glycol (PEG), to the Fc-portion of the human immunoglobulin G (IgG) molecule or to albumin.

Conjugation of PEG-polymers with B-domain deleted rFVIII molecules allowed the production of two fusion products, the site-specific PEGylated BAY 94-9027 molecule with a 60-kDa PEG-polymer (Bayer, Leverkusen, Germany) and the glycoPEGylated N8-GP molecule with a 40-kDa PEG-polymer (Novo Nordisk, Bagsværd, Denmark).[11-13] BAX 855 (Adynovate; Baxalta, Bannockburn, IL, USA) is a PEGylated full length rFVIII with two 20-kDa PEG-polymers and is based on the manufacturing process of the full length rFVIII Advate.[14]

Finally, the site-directed attachment of 40-kDa PEG-polymer to an rFIX molecule generated N9-GP (Refixia; Novo Nordisk).[15,16] The underlying mechanism of the prolonged half-life of the PEGylated molecules is either due to a reduced renal clearance by increasing the volume of the PEGylated product or more likely, given the size of the coagulation factors, to the reduced binding to clearance recep-

tors, such as the low-density lipoprotein receptor-related protein 1.[17]

The extension of the circulating half-life of the rFVIII products by PEGylation is significant but still limited, with a mean half-life between fourteen and nineteen hours (*Table 1*). By contrast, PEGylation of rFIX led to a substantial increase in half-life with a mean half-life of 93 hours (*Table 1*). Overall, good safety and tolerance were demonstrated in clinical trials for BAY 94-9027, N8-GP, BAX 855 and N9-GP.[11-16]

Fusion of the Fc part of a human immunoglobulin G1 with a FVIII or FIX molecule is another bioengineering strategy leading to fusion proteins with extended half-lives. These prolonged half-lives are explained by a recycling mechanism through binding to the neonatal Fc-receptor. The latter is a recycling receptor that protects the Fc-fusion products from lysosomal degradation and releases them back into the circulation (*Figure 1*). Two Fc-fusion products are available, the rFVIII-Fc Elocta (Sobi, Stockholm, Sweden; tradename USA: Eloctate) and the rFIX-Fc Aprolix (Sobi, Stockholm, Sweden).[18-23] Both molecules showed an increase in half-life with mean values around 18.8 and 82.1 hours, respectively (*Table 1*). Good tolerance and safety was observed in clinical trials for both EHL products.[18-23]

According to an analogue mechanism, albumin can also be used to prolong the plasma half-life of the protein to which it is coupled. As IgG, albumin has a naturally long half-life of approximately three weeks and is saved from lysosomal breakdown by a pH-dependent attachment to the neonatal Fc receptor. The fusion product of albumin and FIX (rIX-FP; Idelvion, CSL Behring, Marburg, Germany) has an extended half-life with a mean half-life up to 91.6 hours (*Table 1*).[24-27] Several clinical trials have demonstrated an improved pharmacokinetic profile in comparison with the standard FIX products. Furthermore, rIX-FP is found a safe and effective FIX replacement product for preventing and treating haemorrhages in haemophilia B patients.[24-27]

The much longer half-life extension of the EHL-rFIX molecules compared with the EHL-rFVIII products can be partly explained by the fact that FVIII molecules in plasma are bound to their carrier molecule von Willebrand Factor (vWF). As a consequence, FVIII is cleared by vWF-dependent processes that are not influenced by the modifications of the FVIII molecules.[28]

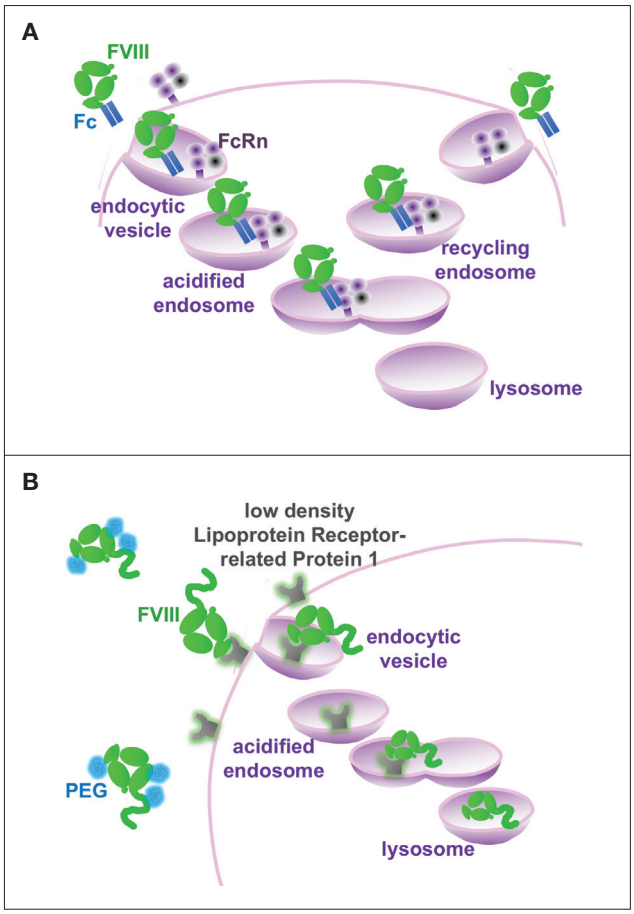

FIGURE 1. Mechanism of extended half-life of FVIII and FIX concentrates. **A.** The fusion of the Fc-domain of human immunoglobulin G1 to FVIII through recombinant technology takes advantage of a biological pathway involving the neonatal Fc-receptor (FcRn). This receptor prolongs the half-life of IgG and albumin, by preventing the degradation of the proteins in the lysosomes and by recycling them to the cell surface. A similar mechanism, mediated by the binding of albumin to FcRn explains the prolonged half-life of albumin-fusion products. **B.** The binding of polyethylene glycolylated recombinant FVIII to clearance receptors, such as the low-density lipoprotein receptor-related protein 1, is reduced by steric hindrance because of the large size of the polyethylene glycol (PEG) moieties.

MONITORING OF EHL RFVIII AND RFIX

The monitoring of coagulation factors with the one-stage assay and the chromogenic substrate assay has always been complicated by discordances in their results. Further difficulties arose with the availability of the first full length rFVIII.[31] Later on, differences up to 50% were reported in the measured FVIII activities of the B-domain deleted rFVIII Refacto and ReFacto AF with the one-stage assay and with the chromogenic substrate assay.[32-34]

The monitoring of the different EHL-rFVIII and -rFIX concentrates has created additional challenges for the haemostasis laboratories. Indeed, each of these novel molecules interacts in a different manner with the assays and reagents available in the clinical laboratories. Many studies have therefore been carried out to assess the appropriateness of the

one stage assays versus the chromogenic assays for the monitoring of the EHL-rFVIII and -rFIX concentrates. Some striking differences between assays can be highlighted. For example, the recovery of BAY 94-9027 is underestimated by more than tenfold with one-stage assays using micronised silica as activator, but can be reliably measured using ellagic acid or polyphenol based one-stage assays.[35] This is not the case for all products. For example, with BAX 855 or with Elocta comparable results for the one-stage assay and chromogenic assay are obtained. Consequently, both assay types are appropriate for post-infusion monitoring of these products.[36] For the measurement of rIX-FP with the one-stage assay, only restricted data are available as most studies were performed with a silica-based reagent. However, some studies reported underestimation of rIX-FP levels with some kaolin and ellagic reagents.[24,37] For the measurement of rFIX-Fc, practically all one-stage assays can be used, except a kaolin based assay that severely underestimates rFIX-Fc activity.[38] Interestingly, EHL-rFIX levels can be accurately analysed with chromogenic substrate assays.[38-40]

Several reviews have addressed this issue and emphasised that the efficient monitoring of the EHL agents will require a good transmission of information between the ordering clinicians and the laboratories.

GENE THERAPY

The recent successful development of FVIII gene therapy has been made possible by modifications in FVIII transcriptional and post-transcriptional processes that were analysed over the last 20 years.[41,42] Codon-optimisation of the FVIII cDNA was particularly important as this modification increases FVIII expression but does not alter the amino-acids of the molecule. The risk of inhibitor development is therefore not increased, whereas the transcription of the FVIII molecule is significantly increased.[42] Other modifications, such as the removal of the FVIII B domain also facilitated FVIII production. In FIX gene therapy, an alternative approach was investigated to increase the FIX levels, the mutation of one amino acid, which increases FIX activity by more than tenfold. This mutation, called FIX Padua, is present in rare patients who suffer from thrombosis rather than bleedings because of the increased FIXa activity.[43]

Following infusion of an adeno-associated viral vector with a liver-specific promoter and a transgene encoding FIX Padua, FIX activity between 14 and 81 IU/dl were measured in plasma of ten participants up to 28 to 78 weeks after administration.[44] The follow-up of these patients is ongoing so that it is still unknown for how long such levels of FIX production will persist. The annualised mean bleeding rates were reduced from 11.1 events per year to 0.4 events per year after administration of the vector. An increase in liver enzymes was observed in a few participants but resolved after glucocorticoid administration.[44]

Therapeutically useful FVIII levels have also recently been achieved with gene therapy in patients with haemophilia A.[45] Following a single injection of adenovirus-associated virus encoding a B-domain deleted FVIII, FVIII activity levels remained higher than 5 IU/dl (5%). In six out of seven treated patients, the FVIII levels were normalised and remained higher than 50 IU/dl up to one year after administration. In this cohort, the bleeding rate was lower than when patients were treated with prophylaxis with FVIII concentrates. The adverse events were limited to a moderate elevation of the serum alanine aminotransferase, which could also be controlled by on-demand or prophylactic administration of glucocorticoids.[45]

BYPASSING AGENTS AND PORCINE FVIII

LONG ACTING RECOMBINANT FACTOR VIIA (RFVIIA)

Bypassing agents such as NovoSeven (Novo Nordisk) and the prothrombin complex concentrate Feiba (Baxalta, Shire) allow the treatment of bleeding episodes in patients with haemophilia and an inhibitor.[9,10] Bypassing agents are also useful to treat bleedings in patients with acquired haemophilia A, a rare bleeding disorder caused by autoantibodies that neutralise FVIII activity.

Bypassing agents can also be used for prophylactic treatment of patients with an inhibitor, although the efficacy of this type of therapy is limited by the relatively short half-life of the products.

To obviate to this shortcoming, Novo Nordisk has developed a 40K glycoPEGylated, recombinant FVIIa (N7-GP). The rFVIIa is coupled to a 40Kd PEG on a N-glycan naturally present in the molecule. Both in healthy volunteers and in haemophilia A patients, the half-life of N7-GP was about 15 hours, significantly longer than the half-life for rFVIIa of 3.5 hours.[46,47] In patients with haemophilia A and an inhibitor, the annualised bleeding rate (ABR) decreased significantly in the treatment period with N7-GP.[48] N7-GP is not yet available in Belgium. A novel, recombinant fusion protein linking factor VIIa with albumin (rVIIa-FP) is also being developed to extend the half-life of rFVIIa.[49]

RECOMBINANT PORCINE FVIII

The recombinant porcine FVIII (rpFVIII; Obizur, OBI-1) is a B-domain deleted rFVIII produced by Baxalta. Given the fact that this rFVIII has the amino acid sequence of the porcine FVIII, it is sufficiently different from human FVIII, especially in the A2 and C2 domains, to prevent most anti-

human FVIII antibodies from neutralising it. Conversely, it is sufficiently homologous to human FVIII to function in the human tenase complex. Obizur has been successfully used to treat patients with acquired haemophilia A, also in cases of unsatisfactory responses to other bypassing agents.[50,51] Interestingly, Obizur has also been used for a high-risk surgery in a paediatric patient with congenital haemophilia A with a high-titer inhibitor to human FVIII.[52] Obizur levels can be monitored with one-stage FVIII assays but not with chromogenic assays, which underestimate the activity of this product by about twofold.[53] The concentrations of antibody to Obizur must also be carefully controlled, because in some cases, possibly dependent on the cross-reactivity of the antibodies, the concentrations may increase within a few days following the initiation of the treatment.[51] Obizur is available in Belgium haemophilia centres since December 2017.

BISPECIFIC ANTIBODY

Emicizumab (ACE910; Hemlibra) is a bispecific antibody developed by Chugai, a subsidiary of Roche. The molecule bridges activated FIX (FIXa) and FX, thereby replacing activated FVIII. Accordingly, the antibody can also restore the coagulation in the presence of FVIII inhibitor antibodies (*Figure 2*).

The usefulness of this antibody has recently been confirmed in a phase III study in patients with haemophilia A and an inhibitor. The annualised bleeding rate was 87% lower among patients treated with a once-weekly subcutaneous administration of emicizumab than the rate among those without prophylaxis. The bleeding rate was also significantly lower than with previous treatment with bypassing agents. The most severe adverse events reported during the trial were thrombotic microangiopathies with thrombosis that developed following treatment with repeated injections of high doses of prothrombin concentrates for bleeding episodes. By contrast, emicizumab was safe when present in conjunction with recombinant factor VIIa. No antibody to emicizumab was detected in this study, although two participants had rapidly reduced emicizumab concentrations over time, suggesting the presence of antidrug antbodies.[55] The antibody has also been successfully used in a phase I/II study in patients with haemophilia A without an inhibitor.[56] In these patients, the administration of the antibody may avoid inconveniences associated with the current treatment of haemophilia A, such as frequent infusion, intravenous administration and FVIII inhibitor development. Emicizumab (ACE910) is used in several clinical studies for patients with or without an inhibitor in Belgium and is currently being registered with the Belgian authorities.

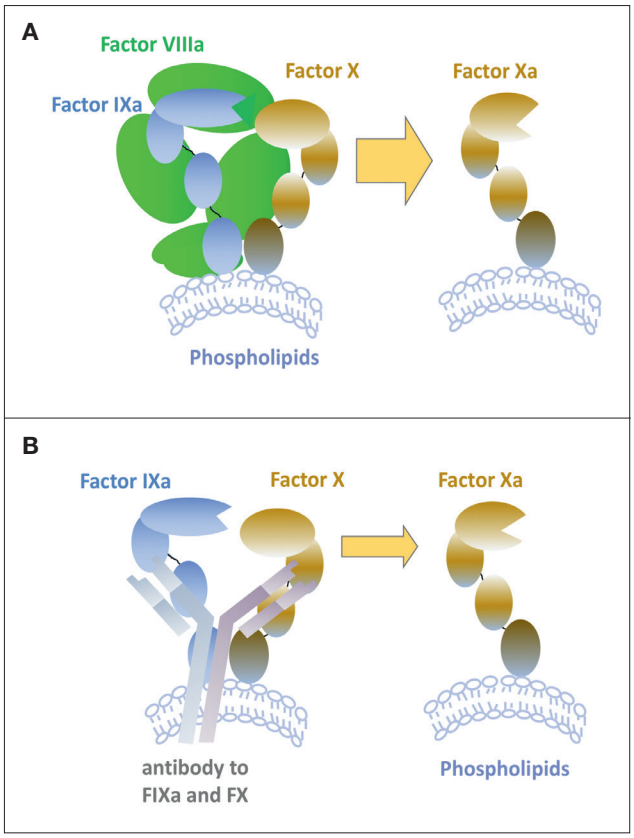

FIGURE 2. Mechanism of action of activated FVIIII and of the anti-FIXa and anti-FX bispecific antibody. **A.** Activated FVIII acts as a cofactor for FIXa in the activation of factor X. **B.** The recombinant humanised bispecific monoclonal antibody emicizumab can replace FVIIIa by binding simultaneously to activated factor IX (FIXa) and factor X (FX).

CONCLUSIONS

Despite the availability of plasma-derived and rFVIII concentrates, the treatment of patients with haemophilia has remained difficult because of the need for frequent administration of the factor concentrates and because of the development of inhibitors, especially to FVIII.

The recent availability of modified rFVIII with a slightly prolonged half-life has provided a modest additional flexibility to the treatment schedules for prophylaxis. By contrast, the development of EHL rFIX has dramatically modified the treatment options for haemophilia B patients by considerably reducing the administration frequency of the FIX concentrates and by allowing higher trough levels. The monitoring of some of these novel recombinant factors must take into account that they interact in different manners with the reagents available in the clinical laboratories for measurement of FVIII and FIX activity. A faultless communication between the clinicians and the laboratory is thus required to avoid any misleading under- or overestimation of FVIII or FIX

KEY MESSAGES FOR CLINICAL PRACTICE

1. The extension of the *in vivo* half-life of recombinant factor VIII (rFVIII) and recombinant factor IX (rFIX) is mediated by coupling the coagulation factors to multimers of polyethylene glycol, to the Fc-portion of human IgG or to albumin. Remarkable prolongation of the half-life is notated for the extended half life (EHL) rFIX molecules, whereas a moderate extension of half-life is observed for the EHL-rFVIII products.
2. Assay related discrepancies were reported in the measurement of the different EHL recombinant factor concentrates. Consequently, efficient post-infusion monitoring of EHL rFVIII and rFIX products requires clear communication between the ordering clinicians and the haemostasis laboratories.
3. The recombinant porcine FVIII Obizur is a bypassing strategy to treat acquired haemophilia A patients with inhibitors.
4. The bispecific monoclonal antibody emicizumab bridges FIXa and FX, thereby replacing activated FVIII. Emicizumab is a novel treatment for patients with hereditary haemophilia A with and without a FVIII inhibitor.
5. Successful gene therapy for haemophilia A and B has recently been achieved.

activity in patients' plasma.

Novel agents useful to restore the coagulation cascade in the presence of antibodies to FVIII or to FIX are expected to facilitate the treatment of patients with an inhibitor. The rpFVIII enlarges the therapeutic arsenal for patients with acquired haemophilia A, whereas the long-acting rFVIIa and the bispecific antibody to FIXa and FX open new perspectives for prophylaxis in patients with haemophilia and an inhibitor.

It is expected that over the next years continued improvement of long-acting factor concentrates, of vector engineering for gene therapy and of therapeutic bypassing agents will further facilitate efficient and convenient treatment for patients with haemophilia A and B.

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