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REVIEW

Emerging drugs for the treatment of hemophilia A and B

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ABSTRACT

Introduction: Replacement therapy with clotting factor concentrates is the most appropriate and effective way to treat bleedings of Hemophilia A&B to prevent chronic arthropathy. Unfortunately, the short half-life (HL) of FVIII/IX concentrates obliges the patients to receive frequent infusions, a big concern for children. The development of inhibitors in about 30–45% of hemophilia A and in 3–5% of hemophilia B patient is the major adverse event of replacement therapy.

Areas covered: In the last few years, new rFIX have been developed with HL. New rFVIII concentrates are displaying small increase of PK characteristics. The new bio-engineering methods allowed the production of molecules fused with Fc fragment of IgG or Albumin or linked to PEG. A new approach to improve hemostasis is represented by Mab against TFPI and small RNA interfering with Antithrombin synthesis. Another innovative drug seems to be the new bi-specific antibody which mimics FVIII function in linking FXa and FX to tenase production.

Expert opinion: The emerging drugs for hemophilia treatment seem to be very promising. The extended half-life will improve the adherence of patients to therapy. Accurate post-marketing surveillance studies will be necessary to check the efficacy, safety and immunogenicity of these new molecules.

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1. Background

Hemophilia A and B are the most prevalent inherited bleeding disorders, due, respectively, to FVIII and FIX deficiency. They account for about 95% of all congenital coagulopathies, being the prevalence of hemophilia A 1/5000 males and that of hemophilia B 1/30,000 males. According to the Global Survey 2014 of World Federation of Hemophilia (WFH), conducted in 106 countries (91% of world population), there are 178,500 hemophilia A and B patients. This figure is definitely underestimated because in underdevelopment countries, the facilities for diagnosis are inadequate or not entire population has been screened. Generally, in these countries, only the patients affected by severe hemophilia are diagnosed at the occurrence of bleedings. Moderate or mild hemophilia can be underdiagnosed in countries with low gross national product (GNP): they are only 11% in Korea or 16% in China with respect to 51% in Canada [1]. As a matter of fact, the hemophilia prevalence ranges from 1/5952 inhabitants in Ireland to 1/873,000 in Nigeria (WFH Annual Global Survey 2014).

The major comorbidity of hemophilia is represented by chronic arthropathy of major joints (ankle, knee, and elbow), triggered by hemarthrosis and skeletal muscle hematomas. In the past, life-threatening bleedings and intracranial hemorrhages reduced the life expectance of these patients. The replacement therapy of missing clotting factor by means of specific plasma-derived or recombinant concentrates is so far the only way to treat these patients, whose life expectance improved very much during the last decades. Even though gene therapy is claimed to be very adapt to cure hemophilia (single gene defect, partial correction of synthesis of clotting factor is enough for a safe hemostasis), new clinical trials on hemophilia are going to start now, after the first disappointing results. The availability of virus-inactivated plasma-derived FVIII (pdFVIII/IX) concentrates after 1986 [2,3] and DNA-derived recombinant concentrates after 1994 [4] made possible the prevention of bleedings or, at least, more appropriate on-demand treatment.

2. Medical need

In the well-developed countries, plasma-derived or recombinant concentrates became more and more available: the average per inhabitant consumption of these drugs increased year by year, notwithstanding the very high cost of these drugs [5]. In 2010, the FVIII use (IU per capita) ranged from 9.2 in Iceland to 0.0077 in India. On the other hand, the treatment of hemophilia in developing countries is so far out of their economic possibilities. As a matter of fact, about 70–80% of hemophiliacs worldwide are undertreated or not treated at all [6]. Generally, there is a relationship between the GNP and the consumption of products for hemophilia treatment [7]. After the success of the virucidal methods against the lipid-enveloped virus (hepatitis C virus [HCV] and human immunodeficiency virus [HIV]), implemented in the manufacturing process of plasma-derived concentrates, the most important undesired side effect of replacement therapy is the development of antibodies against the replaced factor. The prevention of arthropathy can be achieved only by early prophylaxis, started just after the first hemarthrosis and no later than the second year of life [8]. Primary prophylaxis has been showed

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very effective in preventing arthropathy. After the pioneer observations in Sweden and the USA [9,10], two randomized controlled studies [11,12] definitely proved that prophylaxis, compared to on-demand treatment, can significantly reduce the occurrence of joint bleedings. Unfortunately, due to the short half-life of clotting factor concentrates, the need of two to three weekly infusions makes this practice not very easily accepted and performed in children. This is the reason why any improvement of the half-life of concentrates, and therefore any increase of interval between the infusions, is the target of development of new long-acting concentrates.

3. Existing treatment

The virus safety of currently available plasma-derived concentrates is satisfying as far as it regards lipid-enveloped virus because they are very sensitive to the physical (steam or dry heat, pasteurization, and filtration) or chemical (solvent/detergent treatment) inactivation methods. No cases of HIV transmission have been observed among hemophilia patients after 1987 and no HCV-related hepatitis after 1993. Conversely, naked viruses, like B19 Parvovirus and Parvo 4, very resistant to all virucidal methods, are so far contaminating plasma-derived concentrates [13]. The perception of patients about the risk of virus transmission is still very high [14], as well as that of medical doctors [15,16]. The recombinant concentrates are considered as the first-line therapy by many national guidelines. The pd FVIII concentrates have been claimed to be less immunogenic than recombinant ones, even though the screening and follow-up of patients treated with these two different classes of products were generally very different [17–19]. Also European Haemophilia Safety Surveillance did not show any different risk of inhibitors between pdFVIII and recombinant FVIII (rFVIII), and some accurate meta-analysis failed to find a significant odd ratio to prove the worse immunogenic likely of rFVIII [20–22]. Recently, the outcome of a randomized controlled study conducted in previously untreated patients (PUPs) definitely proved that the risk of inhibitor development against FVIII in PUPs treated with rFVIII is 1.87 times than that of patient treated with pdFVIII [23]. Anyway, in this study, only old (first, second, and third generation), full-length, or B-domain-deleted (BDD) rFVIII concentrates have been provided to the patients. The aim of this study was to compare the products by class, recombinant vs. plasma derived, and therefore, no data are available about the difference between the immunogenicity of second- and third-generation concentrates, neither between full-length and BDD ones. Previous prospective studies on rFVIII treatment of PUPs [24,25] have showed a higher incidence of FVIII inhibitor in PUPs treated with a second-generation concentrate. Recently, on 13 May 2016, after an extensive meta-analysis, Pharmacovigilance Risk Assessment Committee stated that currently available evidences do not confirm that second-generation rFVIII concentrates are associated with higher risk of FVIII inhibitors. Anyway, long-term pharmacovigilance on hemophilia patients treated with rFVIII has been recommended by European Medicines Agency (EMA).

4. Market review

The evidence of success of both primary and secondary prophylaxis prompted many hemophilia patients to switch from on-demand to prophylaxis during the last decades. A 10% increase of FVIII consumption has been registered in Sweden each year in the last 40 years [26]. A progressive enhancement of life expectancy and of quality of life made the replacement therapy of hemophilia very much rewarding. Cost-utility and cost-effectiveness studies showed that prophylaxis is cost-effective with respect to on-demand therapy [27]. The incoming new enhanced half-life concentrates will impact on the cost of replacement therapy, but the advantage of fewer venepunctures will made them preferred by parents of children with difficult venous access. Contemporary, the cost of second- and third-generation recombinant concentrates is expected to drop further, according to the experiences of UK national tender [28].

5. Current research goals

Waiting for success of gene therapy, the pharmaceutical companies involved in the hemophilia therapy focused their research and development energies to improve the pharmacokinetics (PK) and pharmacodynamics of currently available recombinant FVIII/IX concentrates. Several bioengineering approaches to improve rFVIII/rFIX manufacturing process have been tried: increased secretion by modifying the posttranslational mechanisms, improved resistance to inactivation, reduced immunogenicity, and extension of half-life. So far, the most rewarding manufacturing process seems to be the extension of half-life, first of all that of rFIX. The claimed reduced immunogenicity of some rFVIII products, a very ambitious aim, must be validated by means of the ongoing studies in PUPs.

6. Scientific rationale

Primary prophylaxis has been proved to be effective in prevention of arthropathy. The secondary or tertiary prophylaxis is not able to reverse chronic arthropathy, but the decrease of joint bleedings may improve the muscle–skeletal conditions and the quality of life of patients, as resulted from extensive studies like Prophylaxis Versus On-demand Therapy Through Economic Report (POTTER) and Trial to Evaluate the Effect of Secondary Prophylaxis With rFVIII Therapy in Severe Hemophilia A Adult and/or Adolescent Subjects Compared to That of Episodic Treatment (SPINART). The need of two to three infusions per week is the principal barrier to primary prophylaxis in children. The venous access difficulties are not always solved by the implantation of central venous catheter because about 50% of them may undergo to infection or occlusion [29]. Consequently, it is easy to understand how the parents of children are eager to use an extended half-life concentrate in order to reduce the number of venepunctures. Of course, a less-demanding regimen of infusions can be attractive also for adults, especially because of rush and stress of modern life. Furthermore, the adherence of adolescents can be improved by less-demanding FVIII/IX concentrates. The other severe side effect of replacement therapy is the development of antibodies against FVIII or FIX. The prevalence of inhibitors against FVIII during the first 50 exposure days (EDs) is quite high, 25–45%, and that of inhibitors against FIX about 3–5%.

Table 1. New recombinant factor VIII concentrates.

Name	Compound	Company	Structure	Indication	Stage of development	Mechanism of action
Kowaltry®	Bay 81–8973	Bayer	FVIII full length	Hemophilia A	Approved by the FDA and EMA	Cofactor of FX activation
Iblias®		CSL Behring	FVIII full length	Hemophilia A	Approved by the FDA and EMA	Cofactor of FX activation
NovoEight®	Turoctocog alfa	NovoNordisk (Bagsværd, Denmark)	BD truncated	Hemophilia A	Approved by the FDA and EMA	Cofactor of FX activation
Nuwiq®	Simoctocog alfa	Octapharma (Lachen, Switzerland)	BD deleted	Hemophilia A	Approved by EMA	Cofactor of FX activation
NovoEight-GP®	Turoctocog alfa pegol	NovoNordisk	BD truncated, site-directed glycopegylation with 40-kDa PEG	Hemophilia A	Phase III completed	Cofactor of FX activation
BAY94–9027	Damoctocog alfa pegol	Bayer	BDD rFVIII, site-specific glycopegylation with 60-kDa PEG	Hemophilia A	Phase III completed	Cofactor of FX activation
Adynovate® (BAX855)	Octocog alfa pegol	Baxalta (Bannockburn, IL, USA)	Full-length rFVIII-controlled pegylation with branched-chain PEG	Hemophilia A	Approved by the FDA 2015	Cofactor of FX activation
rVIII-SingleChain	Ionoctocog alfa	CSL Behring	Single-chain rFVIII compound, B-domain truncated, light and heavy chains covalently linked	Hemophilia A	Phase III completed	Cofactor of FX activation
Eloctate®	Efraloctocog alfa	Biogen (Cambridge, MA, USA)/ SOBI (Stockholm, Sweden)	BDD, Fc fusion	Hemophilia A	Approved by the FDA and EMA	Cofactor of FX activation

FDA: Food and Drug Administration; BD: B-domain; BDD: B-domain deleted; EMA: European Medicines Agency; PEG: PolyEthylene Glycol .

7. Competitive environment

7.1. Recombinant FVIII concentrates

The currently available rFVIII/IX concentrates are derived from Chinese Hamster Ovary (CHO) or Baby Hamster Kidney (BHK) cells that have been transfected with the gene of human FVIII/IX. The first-generation rFVIII concentrates were stabilized by the addition of bovine or human serum albumin either in the cell culture medium or in the final formulation (Recombinate®, Baxter Healthcare International, Chicago, IL, USA); no proteins as stabilizer were added in the final vials before lyophilization of the second-generation rFVIII concentrates (Kogenate®, Bayer Leverkusen, Germany; HelixateNexGens®, CSL Behring King of Prussia, PA, USA; and Refacto®, Pfizer, New York City, USA). The third-generation products lack any added bovine and/or human protein either in cell culture medium or in final formulation (Advate®, Baxter Healthcare International, Chicago, IL, USA; Refacto AF®, Pfizer). Very recently, two new rFVIII concentrates, efraloctocog alfa and simoctocog alfa, have been derived from human embryonic kidney (HEK) cell lines (Table 1).

7.2. New third-generation rFVIII concentrates

7.2.1. BAY 81–8973

BAY 81–8973 is a new full-length rFVIII concentrate of third generation, with the same primary amino acid sequence as sucrose-formulated rFVIII (rFVIII-FS). The FDA and EMA has recently approved BAY 81–8973 (Kowaltry® and Iblias®), and it will soon be available for the treatment of hemophilia A patient. Pharmacokinetic assessments in the LEOPOLD I (Trial to Evaluate the Efficacy and Safety of a New Full Length Recombinant Human FVIII for Hemophilia A) trial showed non-inferiority of BAY 81–8973 vs. rFVIII-FS [30]. In the multinational, randomized, open-label crossover study (LEOPOLD II), severe hemophilia A patients aged 12–65 years were randomized to twice-weekly prophylaxis (20–30 IU/kg), thrice-weekly prophylaxis (30–40 IU/kg), or on-demand treatment with BAY 81–8973. Twice-weekly or thrice-weekly prophylaxis with BAY 81–8973 reduced median annualized bleeding rate (ABR) by 97% compared with on-demand therapy [31]. In another Phase III, multicenter, open-label, nonrandomized study, boys aged ≤12 years with severe hemophilia A and ≥50 EDs to FVIII products received prophylaxis with BAY 81–8973 25–50 IU/kg two times or more weekly for ≥50 EDs. Fifty-one patients were treated (age: <6 years, n = 25; 6 to <12 years, n = 26) with a 2 weekly (43%) or >2 weekly (57%) regimen at study start. Median (Q1; Q3) ABR for the combined age groups was 1.90 (0; 6.02) for total bleeds, 0 (0; 2.01) for joint bleeds, and 0 (0; 0) for spontaneous bleeds. Median (Q1; Q3) ABR of total bleeds within 48 h of previous prophylaxis infusion was 1.88 (0; 3.97) for children aged <6 years and 0 (0; 1.96) for children aged 6 to <12 years. No drug-related serious adverse events (AEs) or inhibitors were reported [32].

7.2.2. Turoctocog alfa (N8)

Turoctocog alfa (N8) is a new B-domain-truncated rFVIII produced by CHO cells. Only 21 amino acids of B-domain are located between the heavy and light chain by a linker that is lost during FVIII activation. All tyrosine sites of molecule are sulfated, and glycosylation is very similar to naive FVIII [33]. The cleaved turoctocog alfa molecules are eliminated during the immunoaffinity chromatography step by means of specific monoclonal antibodies anti-amino acid 740 [34]. *In vitro* studies conducted in hemophilia A blood and preclinical

evaluation in animal models showed a very good efficacy in thrombin generation and clot formation [35]. Bioequivalence between turoctocog alfa and Advate® was shown in a single-dose (50 IU/kg) crossover study in 23 severe hemophilia A patient [36]. A total of 146 adolescents or adults severe previously treated patients (PTPs) participated to a large multinational clinical trial on prophylaxis for 6 months. One or two injections of turoctocog stopped the bleeding in 89% of patients, and the median ABR was 3.7 [37]. No patient developed anti-FVIII inhibitor. Prophylaxis in 63 children with more than 50 EDs (0–11 years old) by 25–50 IU/kg eod (every other day) or 25–60 IU/kg thrice weekly gave rise to similar outcomes: 95% of bleedings controlled with one or two infusion, ABR 3.0, and no inhibitor occurred [38]. A very good hemostatic response was achieved in 33 hemophilia A patients undergoing 15 major and 26 minor surgery procedures [39].

7.2.3. Simoctocog alfa

Simoctocog alfa is a new BD deleted recombinant human FVIII (rhFVIII) concentrates produced by HEK 293 F cells. A plasmid expressing FVIII transfected the HEK cells. No animal or human material was added to the culture medium [40]. Posttranslational glycosylation and sulfation is comparable to that of plasma-derived FVIII. In particularly, simoctocog alfa is devoid of Neu5Gc and α-Gal epitopes, observed in rFVIII concentrates produced by CHO or BHK cells and potentially antigenic to recipients [41]. rhFVIII is purified by affinity resin (VIIISelect) [42]. Virus safety is guaranteed by solvent/detergent method and 20-nm-size pore nanofiltration. The specific activity of rhFVIII is very high, >9000 IU/mg proteins. Simoctocog alfa elicited a normal thrombin generation and was inactivated by activated protein C during *in vitro* experiments. No discrepancy between one-stage clotting and chromogenic assay was observed [43]. The efficacy and safety of *simoctocog* alfa have been proved in a prospective, open-label, Phase III study in 32 adults and severe PTPs during 6 months of prophylaxis, for >50 EDs. Mean ABR resulted 2.28 for all bleeds, 1.16 for spontaneous bleeds, and 1.00 for traumatic bleeds [44]. Similar results have been achieved in a Phase III study in children. Two groups of PTPs, 29 aged 2–5 years and 30 aged 6–12 years, underwent to standard prophylaxis for ≥50 EDs or ≥6 months. According to the preliminary PK study, half-life resulted 11.9 ± 5.4 and 13.1 ± 2.6 in 13 patients of each group. Mean ABR resulted 4.12 for all bleeds, 1.50 for spontaneous bleeds, and 2.34 for traumatic bleeds [45]. Comparing on-demand versus prophylaxis of simoctocog alfa in two different groups of patients but comparable by inclusion/exclusion criteria and demographics, a dramatic decrease of bleedings resulted: 997 bleeds occurred in on-demand group and 36 in patients treated by prophylaxis, and the ABR was 57.44 and 2.30, respectively.

7.3. New-generation long-acting rFVIII concentrates

Different mechanisms have been used to prolong the half-life of factor VIII concentrates:

a. Fusion to fragment crystallizable(Fc) portion of human immunoglobulin 1 (IgG1)
b. Pegylation or glycopegylation
c. Modification of rFVIII molecule: rFVIII-SingleChain

These methods are able to prolong FVIII half-life about 1.4–1.7-fold. The improvements achieved are not so outstanding as those of enhanced half-life rFIX because the half-life of FVIII depends on the clearance (Cl) of endogenous von Willebrand Factor (vWF).

7.3.1. Efraloctocog alfa (rFVIIIFc)

Efraloctocog alfa (rFVIIIFc) is produced in HEK 293 human cells, enabling human glycosylation patterns, as a recombinant BDD factor VIII covalently linked to the dimeric Fc domain of IgG1. Biochemical and functional characteristics are similar to those of other current BDD rFVIII. Fusion of the Fc domain of IgG to an effector molecule prolongs its half-life through endogenous recycling that delays lysosomal degradation of the fusion protein.

7.3.1.1. PK study. The first-in-human, crossover, Phase I/IIa study was conducted in 16 previously treated adult patients with severe hemophilia A (FVIII <1%) who received a single dose of rFVIII (25 or 65 IU/kg), followed by the same dose of rFVIIIFc. The study showed a 1.5–1.7-fold longer elimination half-life (18.8 h for both rFVIIIFc doses versus 12.2 and 11.0 h for 25 and 65 IU/kg of rFVIII), a 1.5–1.6-fold lower Cl, and a 1.5–1.6-fold higher total systemic exposure in patients treated with rFVIIIFc [46]. Dose-dependent peak plasma concentrations and *in vivo* recovery (IVR) were similar, and time to reach 1% FVIII activity was 1.5–1.8-fold longer with rFVIIIFc than with rFVIII across all dosages. The results were confirmed in the Phase III clinical study: rFVIIIFc $t_{½}$ resulted significantly longer, 1.5-fold than that of rFVIII (geometric mean: 19.0 vs. 12.4 h; $P < .001$). The Cl was a 1.5–1.6-fold lower. Time to 1% FVIII trough level above baseline was longer, 1.5-fold for rFVIIIFc than for rFVIII (4.9 vs. 3.3 days, respectively; $P < .001$) [47].

7.3.1.2. Clinical study. About 165 PTPs with severe hemophilia A aged ≥12 years were enrolled in A-LONG (An Open-Label, Multicenter Evaluation of the Safety, Pharmacokinetics, and Efficacy of Recombinant Factor VIII Fc Fusion Protein (rFVIIIFc) in the Prevention and Treatment of Bleeding in Previously Treated Subjects With Severe Hemophilia A), an open-label, multicenter Phase III study designed to evaluate the safety, PK, and efficacy of rFVIIIFc (Table 2). Patients were enrolled into three study arms: (i) individualized prophylaxis (3- to 5-day intervals); (ii) weekly prophylaxis; and (iii) episodic (on-demand) treatment. Median ABR was respectively 1.6 in arm 1, 3.6 in arm 2, and 33.6 in arm 3. Across all arms, 757 bleeding episodes were treated with rFVIIIFc during the efficacy period. Overall, 87.3% of bleeding episodes were resolved with one injection, and 97.8% were controlled with less than or equal to two injections. The median dose per injection to treat a bleeding episode was 27.35 IU/kg. Approximately 30% of subjects achieved a 5-day dosing interval. No subjects developed inhibitors, and rFVIIIFc was well tolerated. The most common AEs (incidence of 5% in the combined arms,

excluding the perioperative period) were nasopharyngitis, arthralgia, headache, and upper respiratory infections [47].

Seventy-one patients were enrolled indeed in Kids A-LONG study, a Phase III open-label study evaluating the safety, efficacy, and PK of rFVIIIFc, in previously treated children with severe hemophilia A aged <12 years [48]. The starting rFVIIIFc regimen was twice-weekly prophylaxis (day 1: 25 IU/kg; day 4: 50 IU/kg). Dose and interval were adjusted based on PK data and clinical assessment with a maximum dose of 80 IU/kg and minimum interval every 2 days. The rFVIIIFc half-life was prolonged relative to that of FVIII, consistent with observations in adults and adolescents. Particularly, rFVIIIFc $t_{½}$ was 12.67 h for those aged <6 years and 14.88 h for those aged 6–12 years. The median ABR was 1.96 overall, and 0.00 for spontaneous bleeds; 46.4% of subjects reported no bleeding episodes on study. Ninety-three percent of bleeding episodes were controlled with one to two infusions. The median average weekly rFVIIIFc prophylactic dose was 88.11 IU/kg. Among subjects who had been previously treated by FVIII prophylaxis, 74% reduced their dosing frequency with rFVIIIFc [48]. Data about the rFVIIIFc extension study, ASPIRE (Long-Term Safety and Efficacy of rFVIIIFc in the Prevention and Treatment of Bleeding Episodes in Previously Treated Participants With Hemophilia A), have recently published. A total of 150 subjects completing A-LONG study, and 61 subjects completing Kids A-LONG study were enrolled in ASPIRE. As of the interim data dead line (6 January 2014), the median time on study was 80.9 (A-LONG) and 23.9 (Kids A-LONG) weeks. The majority of subjects (A-LONG, 92.0%; Kids A-LONG, 57.4%) had ≥100 cumulative rFVIIIFc EDs. No inhibitors were observed. Median ABRs were low with individualized prophylaxis: A-LONG: 0.66; Kids A-LONG <6 years old: 0.0; 6–12 years old: 1.54; weekly A-LONG: 2.03; and modified A-LONG: 1.97. There was no change in prophylactic infusion frequency or total weekly prophylactic dose in the majority of subjects from A-LONG and Kids A-LONG [49].

7.4. PEGylated and glyco-PEGylated rFVIII concentrates

7.4.1. Octocog alfa PEG (BAX 855)

Octocog alfa polyethylene glycol (PEG) (BAX 855) is a PEGylated form (2 moles of a 20 kDa PEG per molecule are bound to surface-exposed lysine) of unmodified recombinant antihemophilic factor, plasma/albumin-free method with the same manufacturing process and biological activity of Advate®. A controlled pegylation process ensures that 60% of the PEG chains are located in the B-domain and are therefore cleaved off upon activation of FVIII [50].

7.4.1.1. PK study. A Phase I, prospective, open-label, crossover, dose-escalation study evaluated safety and PK profile of single doses of BAX 855 compared with single doses of Advate® at two doses. In the 30 ± 3 IU/kg group, subjects were infused with Advate® with collection of seven post-infusion blood samples for FVIII measurement during a 48 h period. After a 72 h washout period, the same dose of BAX 855 was administered with the collection of 14 post-infusion blood samples for FVIII measurement during a 168 h period. The mean half-life and the mean residence time (MRT) of BAX 855 compared with Advate® were 1.4–1.5-fold longer. The same results were confirmed in the pivotal Phase II/III trial conducted in 26 subjects in prophylactic treatment [51].

7.4.1.2. Clinical study. Pivotal Phase II/III, multicenter, open-label study was conducted for the evaluation of efficacy and safety of prophylactic and on-demand treatment of BAX855. Subjects were assigned to treatments on the basis of their prestudy FVIII treatment regimen; however, once 17 subjects were assigned to on-demand treatment (dose 10–60 ± 5 IU/kg for bleeding episode), subsequent 120 subjects were assigned to the prophylaxis (45–65 IU/kg twice-weekly, to maintain FVIII levels >1% based on the results of the Phase I study). Prophylaxis with BAX 855 resulted in an ABR that was significantly lower than half the ABR of on-demand treatment (1.9 versus 41.5; $P < .0001$). About 39.6% of compliant subjects had no bleeding episodes during prophylaxis (57.4% no joint bleeds). Of the 518 bleeding episodes, 95.9% were controlled with one or two infusions at a median dose of 29.0 IU/kg. Treatment was rated excellent/good in the 96.1%. No FVIII-inhibitory antibodies or safety signals were identified [51].

7.4.2. Turoctocog PEG (N8-GP)

Turoctocog PEG (N8-GP) corresponds to rFVIII (turoctocog alfa) PEGylated with a 40-kDa PEG on the *O*-linked glycan in the 21-aminoacid B-domain. After cleavage with thrombin, the activated molecule has the same primary structure as native FVIIIa. The binding capacity of N8-GP to vWF was similar to that of rFVIII [52].

7.4.2.1. PK study. A Phase I clinical trial evaluated safety and PK profile of >12-year-old 26 PTPs with severe hemophilia A (NG8-Pathfinder™ 1) treated with escalating doses (25, 50, or 75 IU/kg) of N8-GP and their previous FVIII product. The incremental IVR after 30 min was 2.5 IU/dL/IU/kg. Dose linearity was observed in the range of 25–75 U/kg. The mean terminal half-life was 19.0 h (range: 11.6–27.3 h), representing an approximately 1.6-fold prolongation compared with patients' previous rFVIII product (mean half-life: 11.7 h). Cl of N8-GP was reduced by approximately 30% compared with the previous product. Volume of distribution (Vd) of N8-GP and that of previous rFVIII was comparable [53].

7.4.2.2. Clinical study. Preliminary results of a Phase III, non-randomized, open-label clinical trial of N8-GP in PTPs >12 years old with severe hemophilia A (NG8-Pathfinder™ 2) have been recently reported in abstract form. In 175 patients treated for prophylaxis with 50 IU/kg of N8-GP, every fourth day, ABR was 1.3, while in the 12 patients on demand was 30.9. Bleeding episode was resolved in 95.5% of cases with one to two injection of N8-GP. One patient developed a neutralizing inhibitor after 93 EDs to N8-GP [54].

7.4.3. Damoctocog alfa pegol (BAY94–9027)

Damoctocog alfa pegol (BAY94–9027) is a modified BDD rFVIII (BDD-rFVIII) which contains a mutated cysteine specifically conjugated to a 60-kDa PEG molecule. The site-specific

PEGylated BDD–rFVIII retained coagulant activity in chromogenic and one-stage assay with ellagic acid as the activator [55].

7.4.3.1. PK study. A Phase I prospective, multicenter, open-label, clinical trial was conducted in 14 patients aged 21–58 years with FVIII of <1%, after that ≥150 days of exposure to FVIII, and no history of FVIII inhibitors. Patients received a single dose of sucrose-formulated rFVIII-FS (seven patients 25 IU/kg [cohort 1] and 75 IU/kg [cohort 2] for a 48 h PK study). After ≥3-day washout, cohort 1 received twice-weekly BAY 94–9027 at 25 IU/kg (16 doses), and cohort 2 received once-weekly BAY 94–9027 at 60 IU/kg (nine doses). A 168-h PK study was performed after the first and last BAY 94–9027 doses. BAY 94–9027 showed equivalent recovery and an improved PK profile vs. rFVIII-FS, with a half-life of ~19 h (vs. ~13.0 h for rFVIII-FS). BAY 94–9027 was well tolerated, and no inhibitor was observed [56].

7.4.3.2. Clinical study. Preliminary results of a Phase II and III, included 132 PTPs >12 years old with severe hemophilia A, have been recently reported in abstract form. Patients received BAY 94–9027 for 36 weeks either on demand or on prophylaxis. Patients were assigned to one of three prophylaxis-dosing regimen based on the number of bleeds observed during a 10-week run-in period, during which all patients in the prophylaxis arm were treated with 25 IU/kg BAY 94–9027 twice/week; in particular, patients with one or less breakthrough bleed were randomized 1:1 to BAY 94–9027 45–60 IU/kg every 5 days or 60 IU/kg every 7 days, while patients with two or more breakthrough bleeds received 30–40 IU/kg BAY 94–9027 twice/week.

Median ABRs for joint due to spontaneous and trauma bleeds were lower for the combined prophylaxis groups (weeks 0–36) compared with the on-demand group (combined prophylaxis groups: 1.5, 1.4, and 0.0, respectively; on-demand group: 16.3, 14.3, and 9.1) [57].

7.4.4. Ionoctocog alfa (rVIII-SingleChain)

Ionoctocog alfa (rVIII-SingleChain) is a novel B-domain-truncated (most of the B-domain and four amino acids of adjacent A3 domain were deleted AA 765–1652) rFVIII, comprised of covalently bonded FVIII heavy and light chains [58]. The activated form of rFVIII (rFVIIIa) produced from rVIII-SingleChain is structurally comparable to that formed from two-chain endogenous FVIII. This novel single-chain design could allow for beneficial features such as high intrinsic stability and molecular integrity, and faster and enhanced binding to vWF (more than threefold higher than full-length rFVIII) may contribute to a low immunogenicity [59,60].

7.4.4.1. PK study. Two studies investigated the PK of rVIII-SingleChain, one in children (<12 years) and the other in adolescents and adults (12 to <18 and 18–65 years) with severe hemophilia A, and the results have been published in abstract form [61]. Subjects received a single infusion of 50 IU/kg rFVIII followed by a single infusion of 50 IU/kg rVIII-SingleChain. PK samples of rVIII-SingleChain were collected prior to infusion (pre-dose) and at 0.5, 1, 4, 8, 10, 24, 32, 48, 72, and 96 h post-infusion in adults and adolescents and prior to infusion (pre-dose) and at 1, 4–6, 10, 24, and 48 h post-infusion in children. In adults and adolescents (n = 35), the mean PK parameters after rVIII-SingleChain dosing were half-life 14.1 h, Cl 2.89 mL/h/kg, and MRT 20.2 h.

As is typical for rFVIII and pdFVIII products, mean half-life of rVIII-SingleChain was shorter in children <12 years compared to adults and adolescents ≥12 years of age (half-life 8.7 h in children <6 years and 10 h in children >6 to <12 years).

7.4.4.2. Clinical study. A Phase I/III study investigated the efficacy and safety of rVIII-SingleChain in the treatment of bleeding episodes, routine prophylaxis, and surgical prophylaxis in PTPs ≥12 years of age with severe hemophilia A (FVIII <1%). Of the 175 patients meeting study eligibility criteria, 173 were treated with rVIII-SingleChain, prophylactically (N = 146) or on-demand (N = 27). The total cumulative EDs were 14,306 days, with 120 participants reaching ≥50 EDs and 52 participants having ≥100 EDs. Hemostatic efficacy was rated by the investigator as excellent or good in 93.8% of the 835 bleeds treated and assessed. Across all prophylaxis regimens, the median ABR was 0.00 (Q1; Q3: 0.0; 2.4), and the median overall ABR was 1.14 (Q1; Q3: 0.0; 4.2). Surgical hemostasis was rated excellent/good in 100% of major surgeries by the investigator. No participant developed FVIII inhibitors [62].

7.5. The new third-generation factor IX concentrates

During the last few years, a plenty of new rFVIII (Table 1) and rFIX concentrates have been developed and started the licencing procedures (Table 2). While third-generation rFVIII concentrates were approved about 15 years ago, nonacog alfa (Benefix®, Pfizer, New York City, USA), the currently available rFIX, was approved for treatment of hemophilia B patients on 1997. At that time, only high-purity pdFIX concentrates were available. Nonacog alfa skipped the first- and second-generation procedures, and it was born as a third-generation product, produced by CHO cells in a cell culture medium free of human or animal proteins. Since the first clinical trials, incremental IVR resulted quite low, 0.73 ± 0.20 IU/dL/IU/kg, definitely lower than IVR of plasma-derived FIX (pdFIX) concentrates [63,64]. On the contrary, half-life 22.4 ± 5.3 h was quite similar to that of pdFIX. Other pharmacokinetic parameters were unknown up to 2001 when the source data of a previous study were analyzed by Björkman et al. [65]. The ranges of Cl and the Vd at steady state (Vdss) among the different age of patients (4–56 years) were evaluated 10.4 ± 2.25 to 7.51 ± 0.26 mL/h/kg and 270 ± 70 to 180 ± 80 mL/kg, respectively. It was evident that the low IVR was determining a low area under the curve (AUC) and a higher Cl. The large Vdss, a characteristic common to all FIX concentrates, was very well explained by the large extravascular distribution of a small molecule like FIX. It is well known that about 40% of infused FIX goes in the extracellular fluids and lymph [66] or binds endothelial cells and collagen type IV in the basement membrane [67,68]. Recently, higher values of IVR, about 1.24 ± 0.32 [69] or 1.21 ± 0.31 [70], were reported probably due to a higher potency of new nonacog alfa formulations. For a very long time, nonacog alfa was the leader of replacement therapy for hemophilia B patients. Even though not licenced for the treatment of children, most of

Table 2. New recombinant factor IX concentrates.

Name	Compound	Company	Structure	Indication	Stage of development	Mechanism of action
Rixubis®	Nonacog gamma	Baxalta	Nonacog alfa	Hemophilia B	Approved by the FDA and EMA	Precursor of FIXa
IXinity®	Trenonacog alfa	Cangene (Winnipeg, Canada)	Nonacog alfa	Hemophilia B	Approved by the FDA	Precursor of FIXa
Innonafactor®	Nonacog alfa	Stragen Pharma SA (Geneva, Switzerland)	Nonacog alfa	Hemophilia B	Approved in Russia	Precursor of FIXa
Idelvion®	Albutrepenonacog alfa	CSL-Behring	rFIX, albumin fusion	Hemophilia B	Approved by the FDA and EMA	Precursor of FIXa
Alprolix®	Eftrenonacog alfa	Biogen/SOBI	rFIX, Fc fusion	Hemophilia B	Approved by the FDA and EMA	Precursor of FIXa
NovoNine GP®	Nonacog beta pegol	Novo Nordisk	rFIX, pegylation	Hemophilia B	Phase III completed	Precursor of FIXa

FDA: Food and Drug Administration; EMA: European Medicines Agency.

the PUPs born after 1997 in the developed countries have been treated off-label with nonacog alfa. As far as efficacy of nonacog alfa was concerned, 82% of 693 hemorrhages resolved after a single infusion and 84% were rated to have good/excellent response, and only 14% moderate responses [71]. After more than 15 years of nonacog alfa monopoly, when the patent was expired, a plenty of biosimilar products have been developed. All these 'me-too' concentrates share the PK/pharmacodynamics (PD) characteristics of original nonacog alfa.

Nonacog gamma (Rixubis®) is produced by Baxalta, and according to a Phase I/III study, it achieved a median ABR of 2.0, and no bleeds occurred in 43% of patients.

Trenonacog alfa (IB1001) was developed by inspiration who took care of Phase I/II crossover study against nonacog alfa, achieving the bioequivalence between these two concentrates [72]. Unfortunately, trace amount of hamster protein resulted contaminating the product and the licence procedure was stopped. At the end, a new purer formulation **(IXinity®)** has been licenced and now produced by Cangene (subsidiary company of Emergent Biosolutions, Rockville, MD, USA). A generic biosimilar of nonacog alfa, **Innonafactor®**, is now produced in Russia by Genrium.

7.6. New-generation long-acting rFIX concentrates

The most outstanding improvements of half-life have been achieved in the manufacturing of three new rFIX concentrates by means of different innovative procedures such as pegylation, Fc, and albumin coexpression (fusion proteins). The first two techniques have been implemented also in rFVIII-manufacturing procedures but with lesser improvement of PK parameters.

Pegylation is a chemical process that allows the binding of linear or branched polyethylene glycol chains to the proteins or peptide. The hydrophilic properties of glycol make the linked drug surrounded by water atmosphere and increase its molecular weight and reduce the kidney Cl or uptake by cellular receptors. Pegylation does not affect drug's stability in a large range of pH and temperature. Glycol is not per se immunogenic, but there are some concerns about the immunogenicity of PEG–protein conjugates [73]. Pegylation increased the half-life of interferon and that of some growth factors. These drugs entered into the clinical practice since many years with good improvement of quality of life of the patients and with clinical success.

Nonacog beta pegol (N9-GP) is the first pegylated rFIX, produced by site-directed binding of branched 40K-PEG to activation peptide of rFIX. The activation peptide with PEG is released during coagulation pathway, and PEG is going to be eliminated by kidney or gastrointestinal tract. In the Phase I/II clinical trial, a single-dose PK study was performed in order to evaluate the dose–response linearity and the PK outcomes of N9-GP in comparison with the currently available plasma-derived or recombinant FIX previously used by 15 enrolled patients [74]. Three groups, five patients each, have been infused with 25, 50, and 100 U/kg. The data analyzed by non-compartmental analysis (NCA) showed a good relationship of AUC, incremental IVR, terminal half-life, Cl, and Vd with administered doses. The timing of collection of blood samples during the single-dose PK of previously used FIX concentrates was stopped at 48 h, while N9-GP PK was prolonged up to 168 h (7 days). Compared to both pdFIX and rFIX concentrates, AUC and Cl of N9-GP resulted about 10 times larger and smaller, respectively, terminal half-life five times longer, and Vd about 50% reduced (Table 3). A *post hoc* evaluation indicated the time of N9-GP to reach 1% and 3% is equivalent to 22.5 and 16.2 days, respectively. The observed smaller Vd of N9-GP is owed to the power of PEG of keeping the factor prevalently in the plasma compartment [74]. The Phase III study (Paradigm 2) on the safety and efficacy of N9-GP enrolled 74 severe, previously treated hemophilia B patients, 59 on prophylaxis by 10 and 40 IU/kg weekly prophylaxis and 15 on demand, for 28 weeks. In the on-demand group, the ABR was 15.58, and in the low- and high-dose prophylaxis group, ABR was 2.93 and 1.04, respectively [75]. As far as the bleeding at level of target joints is concerned, 68% and 8% of patients treated with 40 and 10 IU/kg weekly, respectively, did not experience any bleeding. Similarly, no joints of patients enrolled in the Paradigm 4 extension trial, 40-IU/kg arm, fulfilled the International Society of Thrombosis and Haemostasis criteria for definition of target joints, and the ABR decreased from 8.0 ± 4.3 to 1.32 (0.35–5.05), while in the 10-IU/kg arm, the baseline ABR 5.8 ± 2.5 moved to 4.10 (2.50–6.51) at the end of the trial [76]. Even better results have been achieved during the extension trial Paradigm 4, in which patient from Paradigm 2 and Paradigm 3 (surgery) study were moved. The ABR further decreased to 1.36 and to 1.00 in 10 and 40 IU/kg

Table 3. Summary of outcomes of Phase I/II PK studies of new rFIX concentrates.

	Patients	Dose	Clearance	Half-life	VdArea	Incremental IVR	
Product	*n*	IU/kg	mL/h/kg	Hours	mL/kg	IU/dL/IU/kg	Reference number
N9-GP	8	50	0.71	92.67	95.22	1.34	[74]
pdFIX	8	50	5.48	17.79	140.58	1.12	
rFIX	8	50	6.99	19.34	194.98	0.68	
rFIXFc	5	50	3.44 ± 0.83	77.0 ± 6.80	262 ± 54.2	0.87 ± 0.21	[78]
rFIXFc	22	50	2.8–3.6	71.4–94.5	277.8–356.8	0.77–1.10	[79]
rFIX	22	50	5.6–7.1	29.1–39.2	222.9–305.9	0.81–1.01	
rIXFP	8	50	0.75	91.57	95.0	1.38	[80]
pdFIX	4	50	4.76	14.59	98.7	1.1	
rFIX	8	50	5.24	17.23	130.6	0.94	

IVR: *in vivo* recovery.

arms, and 94.6% of bleedings resolved with N9-GP administration, 87.9% with only one infusion [77].

Albutrepenonacog alfa (rIX-FP) coexpression of rFIX and albumin in CHO cells gave rise to a new molecule, the rIX-FP, where albumin was linked to rFIX by means of a cleavable peptide linker. When rIX-FP undergoes to activation, the link is broken to release albumin [81]. After the good success of the preliminary PK studies in animal models [82], the Phase I/II study was conducted on 25 PTPs [80]. Three doses, 25, 50, and 75 IU/kg, were administered to show the dose–response linearity. The cohort treated with 50 IU/kg underwent to a double single-dose PK with 50 IU/kg of previously used pdFIX (n = 4) and rrIX (n = 8). The blood samples have been collected at 30 min and 48 h post-infusion and only for rIX-FP up to 168 and 336 h, 7 and 14 days, respectively. The data, analyzed by the NCA, showed that incremental IVR of rIX-FP was 29–44% higher, the terminal half-life 5.8–5.3-times longer, and the Cl seven to six times smaller than the corresponding outcomes of rFIX and pdFIX concentrates, respectively (Table 3). The Vd according to terminal half-life (Vz) and at steady state (Vss) resulted 28–30% lower, respectively, than those of rFIX but similar to pdFIX. A sustained 5% trough was achieved 7 days after a 25 IU/kg dose or 14 days after a 50 IU/kg dose. In the Phase III study, 63 PTPs have been enrolled, divided in two groups: one group underwent to weekly prophylaxis for 26 weeks, 35–50 IU/kg, and then switched to longer interval dosing (7, 10, and 14 days, 75 IU/kg) for 50, 38, 51 weeks, and the other group was treated on demand for 26 weeks and after switched to weekly prophylaxis for 45 weeks, 35–50 IU/kg. The patients switched from on demand to prophylaxis experienced about 100% decrease of spontaneous bleedings (ABR range changed from 7.98–17.96 to 0.00–0.96); the rate of complete recovery was 98.6%, and 93.6% of bleedings were treated by a single infusion [83].

Eftrenonacog alfa (rFIXFc) is a protein fusion molecule produced in HEK cells by means of a stable covalent link between rFIX and Fc region of IgG. A plenty of cells are bearing the neonatal Fc receptor (FcRn) which is responsible for long half-life of IgG, about 30 days. Proteins linked to Fc are uptaken by the cells' endocytosis mechanism and afterwards released in the blood stream. The first experiments in normal mice and rats and in hemophilia dogs showed that rFIXFc half-life was three to five times longer than that of rFIX. In the first Phase I/II study, single-dose PK studies (25, 50, and 100 IU/kg) were performed in 11 previously treated severe hemophilia B patients, and a good dose–response was observed [78]. The timing of blood sample collection was prolonged up to 10 days when the dose was 50 IU/kg and to 14 days for patients treated with 100 IU/kg. FIX activity/time data were analyzed by two-compartment method (TCM) because rFIXFc displayed a bi-exponential decay. The range of alfa distribution half-life was 3.31–10.3 h, and beta elimination half-life was ranging from 57.6 ± 8.27 to 56.5 ± 14.1 h. The mean values of Cl resulted 3.18 ± 0.74 mL/h/kg and Vdss 227 ± 57.1 mL/kg Table 3 The design of this study did not demand a comparator group treated with pd-FIX or rFIX, and comparison was made only with literature data. A crossover study comparing rFIXFc against nonacog alfa was performed later, during the Phase III study, in a cohort of 22 severe hemophilia B PTPs before the enrollment in group 1, on weekly prophylaxis with 50 IU/kg [79]. The timing of blood collection of nonacog alfa PK was designed up to 72 or 96 h, while that of rFIXFc up to 240 h. The data were analyzed again with the TCM, and the outcomes of PK confirmed the data of the Phase I/II study (Table 3). Group 2 received prophylaxis with 10-day interval at the beginning, group 3 was treated on demand 20–100 IU/kg, and group 4 underwent surgery under prophylaxis with rFIXFc. ABR decreased by 83% in group 1 and by 87% in group 2. After one dose of 50 IU/kg, the FIX 1% and 3% post-infusion levels were achieved after 11.2 and 5.8 h, respectively. The efficacy of rFIXFc was proved also in the perioperative management of 14 surgeries performed in 12 hemophilia B patients [84]. In this study, FIX activity was predicted by a population PK model of rFIXFc, with a good agreement between observed and predicted FIX activity levels. The decay of rFIXFc seemed to be not affected by surgery. The PK data of Phase III study were used to build a population PK model based on three-compartment model [85] to define the inter-patients, inter-occasions variability and unexplained error of the model in order to simulate the switch of 1000 patients from nonacog alfa to rFIXFc. The study showed that 50 IU/kg weekly and 100 IU/kg every 10 days are able to maintain a steady-state level of FIX activity >1% in 94.5% and 89.2% of patients, respectively.

7.7. New approaches to treatment of bleeding disorders

So far, the replacement therapy of missing factor represents the gold standard of treatment of hemophilia and other congenital bleedings disorders. Notwithstanding, replacement therapy, even by prophylaxis, does not mean 'cure'! Waiting for the very expected success of gene therapy, any new

Table 4. Monoconal antibodies and protein-targeting drugs.

Name	Compound	Company	Structure	Indication	Stage of development	Mechanism of action
ACE 910	Emicizumab	Chugai (Chūō, Tokyo, Japan)/Hoffmann-La Roche (Basel, Switzerland)	Asymmetric bispecific immunoglobulin (Ig) G	Hemophilia A	Phase I/II	It mimics FVIII cofactor
NN 7415	Concizumab	Novo Nordisk	Humanized monoclonal Ig4	Hemophilia A & B	Phase I	Inhibition of TFPI
BAX 499		Baxalta	Aptamer	Hemophilia A & B	Terminated	Inhibition of TFPI
ALN-AT3	Fitusiran	Alnylam (Cambridge, MA, USA)	siRNA	Hemophilia A & B	Phase I	Inhibition of AT

TFPI: tissue factor pathway inhibitor; siRNA: small-interfering RNA; AT: antithrombin.

approach able to avoid the need of frequent venous accesses and the risk of inhibitors will be very well accepted by hemophilia patients and their treaters [86] (Table 4).

Taking into account the hemostasis is the result of a balance between the procoagulant factors and their inhibitors, a new way to improve hemostasis in factor-deficient patients could be achieved by reducing the activity of natural inhibitors. The tissue factor (TF) pathway inhibitor (TFPI) was the first target of this new approach.

Aptamer BAX 499 (formerly ARC19499) is an oligonucleotide able to specifically bind TFPI and to block TFPI inhibition of FXa and of TF–rFVIIa complex. Poor thrombin generation of both hemophilia A & B plasmas can be restored by BAX 499 [87] as well as spatial fibrin formation [88]. A study (NCT01191372) on BAX 499 has recently been stopped because of unexpected bleedings [89].

Concizumab is a monoclonal antibody (mAB2021) specific to second Kunitz domain. TFPI is a single-chain polypeptide synthetized by endothelial cells and comprised of three Kunitz-type domains. The first and second Kunitz domains inhibit the TF–FVIIa complex and FXa, respectively. FXa–TFPI complex in turn shows a strong inhibition of TF–FVIIa complex [90]. Concizumab triggers a procoagulant state *in vitro* and in animal models [91]. Injected in rabbit hemophilia model, mAB2021, as well as rFVIIa, reduced the bleeding time [92]. Biodistribution of Concizumab, investigated by means of immunohistology, is limited to the surface of endothelial cells in rabbits [93]. The bioavailability of Concizumab after subcutaneous administration in nonhuman primates was 93% [94]. Safety of Concizumab was evaluated in normal volunteers and hemophilia patients in a multicenter, randomized, double-blind, placebo-controlled trial. Increasing doses were administered by intravenous and subcutaneous way. Procoagulant activity increased proportionally to the escalated doses [95]. A similar trial (NCT02490787) is now recruiting hemophilia A patients treated subcutaneously with increasing doses of Concizumab.

Fitusiran, a small-interfering RNA (siRNA), is a double-stranded RNA molecule which hampers the expression of specific genes by degrading mRNA after transcription, affecting the protein's translation. A new siRNA targeting the antithrombin (AT) synthesis, ALN-AT3, has been synthetized and tested in animal models [96]. The first Phase I study (NCT02035605), as well as the extension study (NCT02554773), is now ongoing in healthy adult volunteers and hemophilia A or B. Preliminary results showed the safety and efficacy of this drug. A significant increase of thrombin generation in hemophilia plasma has been achieved by AT lowering >75%. Very high ABR (34 ± 10 in 24 patients, AT lowering <25%) decreased to very low values (6 ± 3) in nine of these patients. Potential indications might be predictable for both hemophilia A and B, as well for rare bleeding disorders, because of attractive features of ALN-AT3: stability at room temperature, low volume of administration only monthly, and no antidrug antibody formation.

Emicizumab is one of the several recombinant humanized bispecific and asymmetric IgG antibodies screened to identify the most active one (hBS910) in mimicking the FVIII function, i.e., to link FIXa and FX. hBS910 underwent several steps to improve its FVIII-mimetic activity, PK, purification, and solubility; to remove deamidation sites, and to decrease its immunogenicity. The new investigational name of hBS910 is ACE910. The first *in vitro* experiments showed that ACE910 was displaying a high procoagulant activity in FVIII-deficient plasma and in monkeys, high subcutaneous bioavailability, and 3-week half-life. There was no interference between anti-FVIII antibodies and ACE910, predicting the possibility of its usage also in hemophilia patients with inhibitors [97]. The first efficacy study on bleeding was a controlled trial conducted in nonhuman primates injected with anti-primate FVIII and treated intravenously with recombinant porcine FVIII (10 U/kg twice daily) and ACE910 1 or 3 mg/kg subcutaneously. Bioavailability of ACE910 was 100%, and its half-life was 3 weeks. Both treatments succeeded in restoring a good hemostasis in animals [98]. In the first controlled Phase I study, 64 Japanese or white hemophilia A patients, 11 with FVIII inhibitors, were enrolled. Eight groups, each of six patients, received different doses, according to a dose-escalation scheme, ranging from 0.0001 to 1 mg/kg of ACE910 once-weekly for 4–24 weeks [99]. Two patients per group received placebo. Activated partial thromboplastin time and thrombin generation improved significantly in treated patients. Two out of 48 (4.2%) treated patients resulted positive for non-neutralizing anti-ACE910 antibodies. According to a recent article [100], in three groups of six severe hemophilia A patients each, treated with ACE910, 0.3, 1, and 3 mg/kg once weekly for 12 weeks, the ABR decreased from 32.5 to 2.0, 18.3 to 1.2, and 15.2 to 0, respectively.

8. Potential development issues

The hemophilia treatment scenario is today crowded by many new products, improved traditional concentrates, and innovative ones. In the last 5 years, many new third-generation and enhanced half-life rFVIII/IX concentrates have been

introduced. Modified or new fusion proteins underwent to Phase I/II and III clinical trials, but accurate and validated studies on immunogenicity are still lacking. Recruitment of PUPs is ongoing, and even though some preliminary reports seem to be encouraging, the treaters are waiting for the final outcome. On the other hand, the SIPPET (Survey of Inhibitors in Plasma-Product Exposed Toddlers) study [23] definitely stated the high incidence (44.5%) of inhibitors in PUPs treated with current rFVIII. Therefore, any lower figure of this adverse event will be very well accepted. Long-acting concentrates will allow a longer interval time between bolus administrations, an attracting approach first of all for children. The cost will also be a big issue, first of all in the countries with financial constraints. Physicians will have to try to tailor the right therapy to the patients according to the principle 'one size doesn't fit all.' In the underdevelopment countries, the cheaper plasma-derived concentrates could find a large implementation being safe as never before.

9. Conclusion

The new third-generation rFVIII concentrates are claimed to be less immunogenic. These new rFVIII molecules are BDD or B-domain truncated or single chain. They have been developed in order to reduce the heterogeneity of heavy-chain fragments, after activation of FVIII by thrombin. Avoiding the fragmentation of full-length FVIII in different size isoforms, probably less able to bind vWF, the prevalence of anti-FVIII antibodies elicited by these new rFVIII in PUPs is expected to be less than that (about 45%) achieved by older rFVIII concentrates in the SIPPET study. In addition, improved posttranslational glycosylation made the new third-generation FVIII concentrates more similar to human FVIII. Complete sulfation of tyrosine sites may increase the affinity to vWF, protecting rFVIII from uptake by antigen-presenting cells. This molecule is the driver of FVIII, and therefore, the half-life of new long-acting rFVIII is determined by its natural carrier. This is the reason why the PK outcomes of long-acting rFVIII are not so outstanding as those of new enhanced half-life rFIX concentrates. Replacement therapy of missing factor seems to be questioned after more than 50 years of life by the new approaches aimed to push the hemostatic balance forward thrombosis or by miming the FVIII function. Even though these new drugs deserve big attention, being a monument of ingenuity, a long-lasting surveillance on their AEs after licence will be mandatory.

10. Expert opinion

The limit of FVIII concentrates is the half-life, ranging approximately 8–12 h. As a consequence, frequent infusions are necessary to keep a safe trough, and the adherence of patients to therapy is difficult in some cases. There is no doubt that the new bioengineering technologies improved so much the chemical and biological characteristics of new enhanced half-life rFIX concentrates. On the other hand, the hemostatic efficacy of FIX is not only due to its plasma level. FIX displays a very large extravascular space because about 40% of the injected dose can be found in the lymph and extracellular fluids. FIX binds to the endothelial cell surface and collagen type IV of subendothelial matrix, where also FVIII–vWF complex is located. It is not very easy to assess the role played by FIX in the vessel walls. The relationship between plasma level and hemostatic efficacy of FIX concentrates is not always linearly proportional: PK is only a surrogate test of PD. Taking into account, only half-life could not be enough, being PD related also to other outcomes of PK, as C_{max}, AUC, and, first of all, Cl. In addition, both albumin-fused and Fc-fused new rFIX concentrates are claimed to have an enhanced half-life because of a recycling mechanism. On the contrary, the two concentrates are quite different in terms of half-life, Cl, and Vdss (Table 3) The huge Vdss of rFIXFc may be due to the widespread presence in the body of cells bearing the Fc receptor. The longer half-life and smaller Cl of rIX-FP might be due to a higher rate of recycling of albumin with respect to that of Fc.

Of course, a decreased number of venepunctures will be very well accepted by kids but probably less important to adults. The cost of drug and the financial constrains will play a big role in the definition of appropriateness of these drugs with regard to unmet needs of each patient.

We hope that immunogenicity of new drugs will be soon defined according to EMA recommendations in a large PUPs study. Post-marketing surveillance on this issue is mandatory. As far as the new drugs acting on the hemostatic balance are concerned, careful attention must be paid to the AEs not only in terms of thromboembolic risk but also in terms of immunogenicity. Both monoclonal antibody against TFPI and bispecific antibody mimicking FVIII function are definitely nonself proteins, and the development of antibodies and/or immune complex formation could represent a very dangerous event. Another concern is related to the metabolism and potential toxicity of PEG. Even though PEG has been implemented with success in the production of many other drugs, like interferon, no data are available on its lifelong toxicity. Also immunogenicity of new glyco-PEGylated concentrates must be checked carefully not only in PUPs but even in PTPs, in long-term studies. Again, post-marketing surveillance is mandatory.

Notwithstanding, the treatment of hemophilia, especially of hemophilia B, will improve in the next few years, according to the good results of regulatory clinical trials of enhanced half-life concentrates. The behavior of these new drugs in real life will be probably determined also by patients' features because the bleeding is a multifactorial event. Patient's habits and life styles, age and adherence to the therapy, presence of target joints, and last, but not least, the cost of therapy are some of the causing factors of success of these innovative drugs. For sure, the new long-acting FIX concentrates will determine a big improvement of the quality of life of hemophilia B patients.

The hemophilia treaters should become more familiar with tailoring therapy, especially PK-tailored prophylaxis. It is well known that patients may show a different response to the same concentrate. Since the first studies on PK of FVIII/IX concentrates in 1985 [101], it was evident that the inter-patient variability is huge, but intra-patient or inter-occasion variability is smaller. This is the rationale for tailoring repeated dosing or prophylaxis in hemophilia patients. PK-driven prophylaxis was

shown to reduce the amount of concentrates used for both hemophilia A and B and consequently the cost of treatment. Unfortunately, PK designs are very demanding for young patients, being required several blood samples. To solve this problem, population PK can be very useful, allowing to forecast the FVIII/IX behavior in each patient according to very few blood samples (one or two). Even the switch from an old product to a new one should be driven by PK outcomes. As a matter of fact, even though most of the new rFVIII concentrates resulted bioequivalent with the previous ones, in terms of average half-life, the single patient's IVR, AUC, Cl, and even half-life may be very discordant.

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