A New Era in the Hemophilia Treatment: Lights and Shadows!

Massimo Morfini*
Agency for Hemophilia and Regional Reference Center for Inherited Bleeding Disorders,
Careggi University Hospital, Firenze, Italy

**Corresponding author**
Massimo Morfini, Agency for Hemophilia and Regional Reference Center for Inherited Bleeding Disorders, Careggi University Hospital, Via dello Statuto n.1, I-50129 Firenze, Italy, Tel: 39-348-2306928; Fax: 39-055-473218; E-mail: massimo.morfini@unifi.it

Submitted: 22 August 2016
Accepted: 20 September 2016
Published: 23 September 2016
ISSN: 2333-6684

**ABSTRACT**

At the beginning of 90s’, recombinant FVIII/FIX concentrates were introduced in the hemophilia therapy and in the following 20 years they underwent a progressive improvement of their characteristics, moving from 1st to 2nd and finally to 3rd generation. A new era of recombinant clotting factor concentrates has started since 2010, when new methods were implemented in their production. The most outstanding changes were: improved purification by means of more selective monoclonal antibodies or ligands, human cell lines, co-expression of Albumin or Fragment crystallizable genes, glycopeghylation, molecule modifications as B-Domain deletion and heavy and light chain fusion. These changes caused products with increased Half-life and decreased Clearance, mainly of recombinant FIX and partially of recombinant FVIII, and hopefully an increased pharmacodynamics and a lower immunogenicity. About all phase I/II studies were terminated, some of phase III are still ongoing, and few products have been licensed by Food and Drugs Administration and European Medicine Agency. The hemophilia patients, first of all children, will take advantages from the usage of the Extended Half-Life clotting factor concentrates, being possible a prophylaxis design with weekly or every two weeks infusions. The issue of immunogenicity of new concentrates is now under evaluation by means of studies in Previously Untreated Patients. On the other side of innovative therapies, a new pioneering approach to treatment of bleeding has recently arisen. The down regulation of natural anticoagulants, Tissue Factor Pathway Inhibitor and Antithrombin, by means of specific monoclonal antibodies or small interfering Ribonucleic Acid respectively, increased thrombin generation in FVIII deficient plasma and reduced the Annualized Bleeding Rate of hemophilia patients. Similar results have been achieved by administration of a bi-specific human recombinant monoclonal antibody which mimics FVIII function by linking FIXa and FX.

**ABBREVIATIONS**

AAV: Associated Adenovirus; ABR: Annualized Bleeding Rate; aPTT: activated Partial Thromboplastin Time; AT: Antithrombin; AV: Adenovirus; BD: B domain; BDD: B Domain Deleted; cDNA: complementary Deoxiribonucleic Acid; CFCs: Clotting Factor Concentrates; CHO: Chinese Hamster Ovary; EHL: Extended Half Life; FIXa: activated FX; Fab: Fragment antigen binding; Fc: Fragment crystallizable; FcRN: Fragment crystallizable neonatal receptor; FDA: Food and Drugs Administration; FP: Fused protein; HCV: Hepatitis Virus C; HIV: Human Immunodeficiency Virus; hrMab: human recombinant Monoclonal antibodies; LDL: Low Density Lipoprotein; LTFU: Long Term Follow Up; Mab: Monodonal antibodies; N9-GP: Novo Nine glycopeghylated; PD: Pharmacodynamics; pdCFCs: plasma derived Clotting Factor Concentrates; pdFIX: plasma derived FIX; PEG: PolyEthilen Glycol; PFM: protein Free Material; PK: Pharmacokinetics; PTs: Previously Treated Patients; PUPs: Previously Untreated Patients; rAHF: recombinant AntiHemophilic Factor; rCFCs: recombinant Clotting Factor Concentrates; rFIX: recombinant FIX; rFVIII: recombinant FVIII; SCID: Severe Combined Immunodeficiency; siRNA: small interfering RNA; TF: Tissue Factor; TFPI: Tissue Factor Pathway Inhibitor; UKHCDO: United Kingdom Haemophilia Centres Doctor Organization; VWF: von Willebrand Factor

**INTRODUCTION**

The replacement of missing factor VIII or IX in hemophilia A or B respectively represents so far the key approach to cure the bleeding episodes and to prevent the co-morbidities of these inherited diseases. Since 1964, when Judith Pool [1] serendipitously discovered the cryoprecipitate, the therapy of hemophilia moved the first step toward the care even though not the disease’s eradication. And step by step, pdCFCs became purer and purer. Also the dreadful issue of contamination with blood borne viruses, after the tragedy of the 1975-1985 decade, was at the end successfully answered by implementation of virucidal methods in the manufacturing procedures of CFCs. Lipid enveloped viruses, like HCV and HIV, were completed inactivated by chemical or physical method. After 1987, none

The first era of recombinant concentrates

In 1990, the progresses in cloning FVIII and FIX opened the era of rCFCs. In well developed countries, the vast majority of hemophilia PTPs has been switched from pdCFCs to rCFCs, without any side effects, first all without the development of anti-FVIII/IX antibodies [6]. rCFCs underwent a progressive improvement of their manufacturing characteristics. The 1st generation rCFCs were produced by CHO cell lines growing in a culture medium containing human and animal proteins. To allow the lyophilization, human albumin was added in the final formulation. The 2nd generation rCFCs were lyophylized after the addition of non-protein stabilizers, and in the manufacturing procedures of 3rd generation rCFCs the animal and human proteins of the culture medium were replaced by recombinant ones. In addition, immunoaffinity purification of FVIII by means of Mab was replaced by ion exchange chromatography by means of synthetic ligand (Refacto AF®).

The second era of recombinant concentrates

In the interval between 1990 and 2010, 5 new rFVIII/IX concentrates have been developed and licensed. In the last six years, 10 new rFVIII/IX concentrates entered phase I/II/III clinical trials and some of them have already been licensed in USA and EU. This outstanding increase is due to the efforts of pharmaceutical company to face the unmet needs of hemophilia patients. The first impediment to preventive treatment of bleedings in children (primary prophylaxis) is represented by difficulties of venous access. Primary prophylaxis has been shown to be able to prevent hemophilia arthropathy by well-designed randomized clinical trials [7,8]. Therefore, any effort of pharmaceutical companies was aimed to increase the permanence of infused factor in blood stream. Several methods have been developed to achieve this aim that can be summarized in three groups: 1-Modification of molecule structure; 2-Pegylation of the molecule; 3-Co-expression of genes of Albumin or Fc.

New rFVIII with improved half-life

The most common method of producing recombinant FVIII is now the B-Domain-Deletion. The intra-cellular flow of BDD FVIII seems to be faster and the yield higher than that of full-length rFVIII. As a matter of fact, about all new rFVIII concentrates is BD deleted concentrates, being the heavy chain and the light chain fused by a covalent link [9]. A new BD rFVIII concentrates was co-expressed together with Fc (rFVIII-Fc) in Human Embryonic Kidney (HEK) cells in order to take advantages of recycling cellular process through the FcRn [10]. Really this concentrate showed a half-life about six hours longer than the standard ones but very similar to that of two PEGylated concentrates, the BAY94-9027 [11] and N8-GP [12] (Table 1). On the other hand, the in vivo behavior of FVIII is determined essentially by the turnover of its carrier, the VWF. Even though pegylation is able to decrease the uptake of FVIII by hepatic cells exposing the LDL receptor, the effect of this procedure has had a limited effect on FVIII in vivo decay. Another B Domain deleted rFVIII was produced in HEK cells [13] with improved half-life [14] (Table 1).

Efficacy and safety of new EHL FVIII concentrates has been evaluated in phase III studies. In the A-Long trial of rFVIII-Fc large cohort of severe PTPs (n=118) underwent individualized prophylaxis 25-65 IU/kg every 3-5 days rFVIII-Fc (Arm 1), while other 47 were randomized in two arms, weekly prophylaxis by 65IU/kg (Arm 2; n=24) and on demand treatment, 10-50IU/kg (Arm 3; n=23) [15,16]. ABR was 33.6 in the Arm3, 3.6 in Arm 2 and 1.6 in Arm1. The 89.6% of bleeding episodes have been treated with 2 injections, 87.3% with only 1 injection. Kids A-Long study [17] showed that twice-weekly injections, well tolerated by children, yielded low bleeding rates. In the extension study ASPIRE the median ABR was 18.36 in the on demand group (n=14), 2.03 in the weekly prophylaxis, and 0.66 in the individualized prophylaxis [18].

A 20 KD aPEGylated recombinant full-length anti-hemophilic factor (rAHF) plasma-albumin free method (PFM) [19] (BAX 855) showed normal biological activities and a half-life about 3 hrs, longer than that of Advate®. According to the study comparing on demand vs prophylaxis treatment, ABR decrease from 41.5 to 1.9 respectively, a 95.4% reduction. In this study, BAX855 half-life resulted 4 hours longer than that of Advate® [20]. Pathfinder™ study evaluated the safety and efficacy of turoctocog alfa pegol (N8-GP) in PTPs > 12 years old. A small group (n=12) of patients have been treated on demand and a large group (n=175) by prophylaxis, 50IU/kg every 4 days. The ABR resulted 30.9 and 1.3 respectively, a 95.8% decrease [21].

BAY 94-9027 showed equivalent recovery and an improved PK profile vs. rFVIII-FS, with a half-life average of about 19 h (vs. 13.0 h for rFVIII-FS). BAY 94-9027 was well tolerated, and no immunogenicity was observed [11]. In the BAY 94-9027 Protect study, patients were randomized to two prophylaxis regimens: 45-60 IU/kg every 5 days or 60 IU/kg every 7 days. In the on demand arm, ABR was 23 and fallen to 4.1 in the twice/weekly group, 1.9 in every 5 days` group and 3.9 on the group of weekly prophylaxis [22]. In the Protect VIII kids trial, the ABR dropped to 2.87 [23].The half-life of FVIII single-chain resulted a bit longer than that of Advate® in a head-to-head study [24] (Table 1). The hemostatic efficacy was rated excellent/good in 93.8% of 835 bleedings treated with in average 32 IU/kg during the phase III study. Total ABR dropped from 19.64 in the on demand arm (n=27) to 1.14 in all patients on prophylaxis (n=146) [25].

New rFIX with extended half-life

The implementation of co-expression of Albumin (Albutrepenonacog alfa) and pegylation of rFIX (Nonaoc bgatepegol) determined the most extraordinary results in improving the half-life of both these factors (Table 2). 5 and 6 times longer than that of pdFIX and nonacog alfa respectively,
According to the head-to-head comparative PK studies of phase I/II [26,27], less evident increase of FIX half-life has been achieved by co-expression of Fc (Eftrenonacog alfa) [28,29]. Taking into account only the three head-to-head phase I/II studies, the clearance of the EHL rFIX concentrates resulted respectively about 10.7 and 2 times smaller than that of nanocog alfa (Table 3). The half-life of nanocog beta pegol and albutrepenonacog alfa resulted about 5 times longer than that of nanocog alfa (Table 3). These impressive data are partially biased by the different sampling design of PK adopted in the two studies, shorter for pdFIX and its half-life resulted quite long, as also in other PK studies using sampling times of nonacog alfa was prolonged up to 72 hrs and resulted 18.0 in on demand group and dropped to 3.0 and 1.4 in the once weekly and tailored prophylaxis arms respectively [29]. In the B-Long study, 63 patients have been treated once weekly with 50IU/kg as starting dose, afterward adjusted by PK outcomes and29 patients underwent to tailored prophylaxis (10IU/kg (30 patients) and 40 IU/kg (29 patients) was 2.0 and 1.0 respectively, an 81.4% reduction. Even more outstanding outcomes have been observed in phase III study of albutrepenonacog alfa where three groups of patients have been treated by prophylaxis with 40 IU/kg or 75 IU/kg weekly or every two weeks, respectively. In the on demand group the median ABR resulted 15.43 and dropped to 0.0 in all groups on prophylaxis [33]. In the B-Long study, 63 patients have been treated once weekly with 50IU/kg as starting dose, afterward adjusted by PK outcomes and 29 patients underwent to tailored prophylaxis (100IU/kg every 10days at the beginning) to target 1-3 IU/dL. The ABR resulted 18.0 in on demand group and dropped to 3.0 and 1.4 in the once weekly and tailored prophylaxis arms respectively [29].

### New approaches to treatment of bleeding disorders

The blood clotting is the results of a balanced action of agonist (procoagulant) factors and antagonist ones (natural anticoagulant). When the procoagulant factors dominate the anticoagulant ones, the thrombosis occurs. In the large majority of cases the bleeding is caused by an unbalance of the system, due to a deficiency of procoagulant factors or by an excess of acquired or natural anticoagulants. In order to rebalance the system of inherited bleeding deficiencies, we can try to reduce the action of natural anticoagulant factors. This mechanism is on the basis of action of Mab inhibiting the TFPI and of siRNA decreasing the hepatic synthesis of Antithrombin (Table 4).

Emicizumab (ACE910) is a human recombinant IgG antibody with one Fab specific for FIXa and the other for FX. Linking and allowing special interaction of these two principal actors for...
Table 3: Ratios between clearance of pdFIX or rFIX EHL rFIX and Half-life of EHL rFIX and pgFIX or rFIX.

<table>
<thead>
<tr>
<th>Product</th>
<th>Clearance (mean)</th>
<th>Clearance ratios pdFIX/ or rFIX/EHL FIX</th>
<th>Half-life (mean)</th>
<th>Half-life ratios EHL FIX/ or EHL FIX/rFIX</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mL/h/Kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonacog alfa pegol</td>
<td>N9-GP 0.71</td>
<td>pdFIX/ N9-GP 7.7</td>
<td>17.79</td>
<td>5.21</td>
<td>27</td>
</tr>
<tr>
<td>pdFIX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nanocog alfa</td>
<td>rFIX 6.99</td>
<td>rFIX/N9GP 9.8</td>
<td>19.34</td>
<td>4.79</td>
<td>29</td>
</tr>
<tr>
<td>Eftrenonacog alfa</td>
<td>rFIX-Fc 3.20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nanocog alfa</td>
<td>rFIX 6.30</td>
<td>rFIXFc 2.0</td>
<td>34.15</td>
<td>2.43</td>
<td></td>
</tr>
<tr>
<td>Albutrepenonacog alfa</td>
<td>rIX-FP 0.75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pdFIX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: pdFIX: plasma derived FIX; rFIX: recombinant FIX; Fc: Fragment crystallizable; N9-GP: Novo nine glycopeghylated; FP: Fused protein; EHL: Extended Half-Life

Table 4: Drugs targeting procoagulant and anticoagulant plasma clotting factors.

<table>
<thead>
<tr>
<th>Name</th>
<th>Compound</th>
<th>Company</th>
<th>Structure</th>
<th>Indication</th>
<th>Stage of development</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE 910</td>
<td>Emicizumab</td>
<td>Chugai/Hoffmann-La Roche</td>
<td>Asymmetric bispecific IgG</td>
<td>Haemophilia A</td>
<td>Phase I/II</td>
<td>ACE910 mimics FVIII co-factor</td>
</tr>
<tr>
<td>ALN-AT3</td>
<td>Fitusiran</td>
<td>Alnylam</td>
<td>siRNA</td>
<td>Haemophilia A&amp;B</td>
<td>Phase I</td>
<td>Inhibition of AT</td>
</tr>
<tr>
<td>NN-7415</td>
<td>Concizumab</td>
<td>Novo Nordisk</td>
<td>Humanized monoclonal IgG</td>
<td>Haemophilia A&amp;B</td>
<td>Phase I</td>
<td>Inhibition of TFPI</td>
</tr>
</tbody>
</table>

Abbreviations: AT: Antithrombin; TFPI: Tissue Factor Pathway Inhibitor; siRNA: small interfering RNA

tenase production, Emicizumab mimics the FVIII function. In vitro test showed that ACE910 is able to substitute FVIII in deficient plasma [34]. In an animal model of acquired deficiency of FVIII (non-human primates depleted of FVIII by means of a specific anti-FVIII antibody), subcutaneous administration of ACE910 1-3 mg/kg restored normal hemostasis [35]. The first phase I was conducted in a large population of Japanese and Caucasian (n=64, treated 48, placebo 16 patients), 17% of them with anti-FVIII inhibitors. Eight groups of patients (each n=6 patients) were treated weekly for 4-24 weeks with single, escalating doses of ACE910, ranging from 0.0001 to 1 mg/kg. A significant reduction of a PTT and increase of thrombin generation were observed [36]. Two patients (4.2%) developed a non-neutralizing antibody against ACE910. Very outstanding results on the efficacy of ACE910 derived from a phase III study: in three groups of hemophilia patients treated with ACE910 0.3, 1, and 3 mg/kg once weekly, the ABR dropped to 2.0, 1.2, and 0.0 from very high values (32.5, 18.3, and 15.2) respectively [37].

Fitusiran (ALN-AT3) is a siRNA recently developed [38] to suppress the hepatic synthesis of Antithrombin. ALN-AT3 is able to increase thrombin generation in hemophilia plasma when the Antithrombin concentration is reduced up to 25%. The first phase I study is still ongoing and preliminary results are showing a significant reduction of ABR, from 24-44 to 3-9 in hemophilia patients, who’s AT concentration was lowered to 25% [39].

Concizumab is a Mab IgG 4 inhibiting both 1 and 2 Kunitz domain of TFPI, being these domains the strong inhibitors of FVIIa TF complex and FXa respectively, the most important triggers of hemostasis mechanism. In vitro studies showed that Concizumab was able to decrease the prolonged a PTT of hemophilia plasma [40]. In rabbit hemophilia model Concizumab administered subcutaneously reduced the skin bleeding time and loss [41]. High bioavailability by subcutaneous injection has been reported in the monkey studies [42]. An escalation dose finding study, phase I, showed a good dose/procoagulant response [43]. The trial NCT02490787 (Trial Investigating Safety, Pharmacokinetics and Pharmacodynamics of Concizumab Administered Subcutaneously to Hemophilia A Subjects) is now ongoing and recruiting patients to be treated subcutaneously with increasing doses of Concizumab.

Gene Therapy

Hemophilia A or B are both monogenic inherited diseases, very well characterized during the end of last millennium. Even

Table 5: A comparison between outstanding improvements of half life of new EHL rFIX concentrates and the modest one of new EHL rFVIII concentrates.

<table>
<thead>
<tr>
<th>New EHL rFVIII concentrates</th>
<th>Half-life (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N8-GP</td>
<td>19.0</td>
</tr>
<tr>
<td>BAY94-9027</td>
<td>19.0</td>
</tr>
<tr>
<td>rFVIIIFc</td>
<td>18.8</td>
</tr>
<tr>
<td>BAX855</td>
<td>16.0</td>
</tr>
<tr>
<td>FVIII Single Chain</td>
<td>14.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>New EHL rFIX concentrates</th>
<th>Half-life (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N9-GP</td>
<td>92.67</td>
</tr>
<tr>
<td>rFIXP</td>
<td>91.57</td>
</tr>
<tr>
<td>rFIXFc</td>
<td>77.0</td>
</tr>
</tbody>
</table>
though the correlation between clinical phenotype and plasma factor level is not very strict (some patients are laboratory severe and clinically mild), patients able to produce more than 1-3 IU/dL are experiencing less frequent and milder bleedings. FIX gene was cloned on 1982 [44] but only two years later also FVIII was isolated and characterized [45]. These findings opened the way not only to the production of recombinant clotting factors but also to the first attempts of hemophilia gene therapy in hemophilia animal models, mice and afterwards dogs. Due to the smaller size of FIX, about 1.4Kb with respect to FVIII, approximately 4.4 Kb, it was easier to find a vector able to transfer FIX gene into target cells. The aim of gene therapy in hemophilia is the expression of active clotting factor at minimum level to avoid bleeding and in a long term way, possibly lifelong. The best candidate for normal gene transfer was identified in Retrovirus, [46] able to transfect randomly and only the replicating cells (phase S) with very high efficiency and long term expression. A modified HIV Lentivirus [47] can transfect cells in phase G0, with good but transient expression of transferrered gene, due to a strong immune response. Gammaretroviral vectors were used to cure some children affected by SCID with good clinical success. Unfortunately, after 3-6 years 4 out 9 patients in [48-50] developed T-cell acute lymphoblastic leukemia.

AV and AAV drew the attention of investigators. Long term expression of FIX was achieved after intramuscular injection of recombinant AAV in mouse [51] and afterwards also in hemophilia dogs [52]. Unfortunately, when this procedure was tried in hemophilia B patients, the quite good FIX level achieved at the first, declined to 0.5-1.5 IU/dL after one year [52]. To improve FIX expression, AAV infusions were performed directly in the hepatic artery [53]. Bio distribution studies showed the presence of vector DNA in the seminal fluid even not in spermatozoids. After adopting some warnings for patients (barrier birth control, sperm banking), the study was resumed. Very good FIX level (12 IU/dL) was elicited by high-dose administration but post-infusion liver enzyme peak was followed by decrease of FIX concentration to baseline value [54]. This liver toxicity was due to the immune response to vector capsid peptides, present at the surface of transfected liver cells [55]. AAV infection is community acquired in a plenty of people and antibodies against the different AAV serotypes are present in about 40% of population [56].

In order to accept the large FVIII cDNA, part of AAV have been removed: the “gutless” AAV was infused by i.v. way in three patients who achieved 3 IU/dL FVIII level. Higher FVIII concentration resulted after higher doses but with severe side effects (thrombocytopenia and elevation of transaminases) [57].

Ex vivo approach was adopted to insert in patient’s cultured fibroblasts the B Domain Deleted (BDD) FVIII cDNA contained in a plasmid. Transfected fibroblasts were re-inserted in the patient omentum by endoscopic surgery. After initial success (FVIII level 4 IU/dL) in 4 of 6 patients treated, one year later the FVIII expression was lost [58].

The most outstanding progresses in hemophilia gene therapy have been achieved in the 3rd millennium. In London, at Royal Free Hospital, six hemophilia B patients have been infused with AAV8 carrying hFIX: they developed plasma FIX level between 2 and 11 IU/dL for 6.16 months [59]. Some of them stopped weekly prophylaxis and did not experience any bleedings; other reduced the total rFIX consumption. Two patients received a very high dose of vector and developed transaminases elevation, promptly controlled by steroids. Follow up of 10 hemophilia B patients, treated with a single infusion, and showed that after more than three years a stable FIX plasma concentration ranging from 6 to 1 IU/dL. The incidence of bleedings per year decreased by 90% after the gene transfer with respect to the previous period [60].

Following these successes, eight clinical trials on hemophilia B gene therapy are now approved by FDA but only five are recruiting patients. The only one clinical trial on hemophilia A, started on 2015, has now suspended recruitment of patients, even still ongoing according to Clinical.trial.gov (NCT02576795).

Notwithstanding the good outcomes of gene therapy in the last five years, the pathway to a successful gene therapy seems to be very long. The expression of protein by transfected cells seems to be quite valid but the side effects, first of all insertion mutagenesis and liver immune mediated damage seem to be the major impediments to wide implementation of gene therapy in the hemophilia treatment scenario. Recently, the development of human hepatocellular carcinoma has been related to AAV2 vector [61]. So far, no gene therapy protocol has been licensed for the marketing. FDA recommended recording carefully LTFU for at least 15 years [62]. Taking into account that viral vectors are always non-self proteins, seroconversion against their epitopes prevents a new gene therapy treatment with the same vector; repeated infusions must be done with new, not cross-reacting viral vectors. Recently Cochrane reported that the advantage of gene against replacement therapy has not been proved by any randomized controlled trials [63].

A new approach to correct the gene defect of hemophilia patients seems to be the correction of mutation present in their X chromosome instead of transferring a normal gene in the cells. Direct gene targeting is the base for genome editing, now improved by using zinc finger nucleases, able to recognize a specific DNA sequence. A clinical trial named “Ascending Dose Study of Genome Editing by the Zinc Finger Protein (ZFP) Therapeutic SB-FIX in Subjects with Severe Hemophilia B” has been approved but not recruiting patients (NCT02695160).

**DISCUSSION & CONCLUSION**

There is no doubt that the hemophilia treaters are living a new and exciting era of treatment because the appearance on the scenario of new and improved recombinant CFCs. The extension of half-life, very impressive as far as the new rFIX concentrates is concerned, will allow adopting longer intervals in the prophylaxis regimen. The dream of each patient is to be completely free of bleeding with the minimal number of infusions. Every two weeks infusion seems to be realistic for hemophilia B patients. Even a shift from three to two time’s weekly infusion may be significant for a child, meaning a decrease from 180 to 120 infusions per year [64]. Also the adherence to therapy of adolescent or adult patients will be improved by the availability of new EHL CFCs. We can predict that the majority of patient will ask to be switched from current recombinant to the new EHL CFCs. The reduced clearance of EHL CFCs will allow reduced doses for repeated prophylactic home or even peri-operative treatment. Given the similar PD
and efficacy of new EHL CFCs with respect to the older ones, we can
guess that the cost of new products will be higher. We hope
that the increase of cost will not be inversely proportional to the
decrease of doses. In this case, the financial crisis of EU will make
difficult the widely usage of EHL CFCs for all hemophilia patients.
The switch from old to new CFCs should be managed on the
basis of scientific criteria and recommendations. UKHCD0 issued
recently some guidelines about this issue [65]. A PK driven switch
seems to be the most rational approach to the change of therapy.
This procedure could allow two evaluate the difference between
the PK outcomes of old and new concentrates. The comparison is
very useful to show the differences to the patient or his parents
but first of all to tailor the infusion regimen according to the PK
results. The immunogenicity and safety of new EHL CFCs are not
so far very well known. Even though Albumin and Fc are self
proteins, the new bio-engineered fusion factors might be non-
self. This does not seem to be the case, because in all phase I/II
III studies conducted in PTPs, none inhibitor developed. The final
answer will be provided by studies now ongoing in the PUPs. PEG
toxicity is another concern. A plenty of other PEGylated drugs
are on use since many years for short term treatment of different
diseases without any toxic effect but hemophilia treatment
requires a long life replacement therapy. Long term prospective
pharmacovigilance studies will be needed to definitely answer
this issue.

The new alternative therapies for the management of
hemophilia patients with or without inhibitors deserve a careful
evaluation. The deregulation of natural inhibitors, like TFP1 and
AT, seems to be able to rebalance the haemostatic mechanism
but some concerns have been arisen about the thrombo-
embolic risk of this approach. Careful finding dose studies and
recommendations will be needed. The development of bispecific antibody ACE910 is a monument
of ingenuity. If the dramatic decrease of ABR will be confirmed
in large size studies, the treatment of patients with inhibitors
will find a final and safe solution. The way of administration
of all new alternative drugs is subcutaneous. The patients will
appreciate very much avoiding the i.v. administration but we
must remember that subcutaneous way is the best to stimulate
the immune system. Emicizumab and Concizumab are Mabs
which work by non-clotting. When high frequencies of exposure to the novel human parvovirus PARV4


following a major switch from full-length to B-domain-deleted factor VIII: a prospective cohort comparison. Haemophilia. 2015; 21: 219-
226.


REFERENCES


bleeding disorders treated with plasma-derived factor concentrates in the era of nucleic acid test screening. Transfus. 2013; 53: 1217-
1225.


following a major switch from full-length to B-domain-deleted factor VIII: a prospective cohort comparison. Haemophilia. 2015; 21: 219-
226.


Development, upscaling and validation of the purification process for
human-cl rhFVIII (NovoNordisk®), a new generation recombinant factor VIII
produced in a human cell-line. Protein Expr Purif. 2015; 115:165-175.

human cell line-derived recombinant factor VIII (Human-
cl rhFVIII, NovoNordisk®) in children with severe haemophilia A: efficacy,
safety and pharmacokinetics. Haemophilia. 2015.

15. Shapiro AD, Ragni MV, Kulkarni R, Oldenburg J, Srivastava A, Quon
DV, et al. Recombinant factor VIII Fc fusion protein: extended-
dose interval dosing maintains low bleeding rates and correlates with von

I, et al. Phase 3 study of recombinant factor VIII Fc fusion protein in

Recombinant factor VIII Fc fusion protein for the prevention and
management of bleeding in children with severe hemophilia A. J Thromb

term safety and efficacy of recombinant factor VIII Fc fusion protein
(rFVIII-Fc) in subjects with haemophilia A. Haemophilia. 2016; 32:
1-12.

M, et al. BAX 855, a PEGylated rVIII product with prolonged half-

20. Konkle BA, Stasyszyn O, Chowdary P, Bevan DH, Mant T, Shima M,
et al. Pegylated, full-length recombinant factor VIII for prophylactic
and on-demand treatment of severe hemophilia A. Blood. 2015; 126:
1078-1085.

Lentz SR, et al. Clinical evaluation of novel recombinant glycoPEGylated
FVIII (turoctocog alfa pegol, N8-GP): efficacy and safety in previously
in treatment with patients with severe hemophilia A - results of pathfinder®

22. Boggio LN, Hong W, Wang, Eyster ML, Michaels LA. Bleeding
Phenotype with Various Bay 94-9027 Dosing Regimens: Subanalyses

Protect VIII Kids trial results: BAY 94-9027 safety and efficacy in
previously treated children with severe haemophilia A. Haemophilia.

24. Klamroth R, Simpson M, von Depka-Prondzinski M, Gill JC, Morfini M,
Powell JS, et al. Comparative pharmacokinetics of rVIII-SingleChain
and octocog alfa (Advate®) in patients with severe haemophilia A.
Haemophilia. 2016.

25. Mahlangu J, Kulickowski K, Karim FA, Stasyszyn O, Kosinova MV,
phase 1/3 multicenter clinical trial in severe hemophilia A. Blood.
2016; 128: 630-637.

I, Voigt C, et al. Safety and pharmacokinetics of a novel recombinant
fusing protein linking coagulation factor IX with albumin (rIX-FP) in

pharmacokinetic properties of a glycoPEGylated recombinant factor
IX: a first human dose trial in patients with hemophilia B. J Thromb
Hemost. 2011; 118: 2695-2701.

28. Shapiro AD, Ragni MV, Valentino LA, Key NS, Josephson NC, Powell JS,
et al. Recombinant factor IX-Fc fusion protein (rFIXFc) demonstrates
safety and prolonged activity in a phase 1/2a study in hemophilia B

29. Powell JS, Pasi RJ, Ragni MV, Ozelo MC, Valentino LA, Mahlangu JN,
et al. Phase 3 study of recombinant factor IX Fc fusion protein in

A, et al. Head-to-head comparison of the pharmacokinetic profiles of a
high-purity factor IX concentrate (AlphaNine®) and a recombinant
factor IX (BenefIX®) in patients with severe haemophilia B.

MG, et al. Correlation between FIX genotype and pharmacokinetics
of Nonacog alpha according to a multicentre Italian study. Haemophilia.

32. Collins PW, Young G, Knobe K, Karim FA, Anghaiasukiri P, Banner C,
et al. Recombinant long-acting glycoPEGylated factor IX in hemophilia
B: a: a multinational randomized phase 3 trial. Blood. 2014; 124:
3808-3868.

33. Santagostino E, Martinowitz U, Lisitschikov T, Pan-Petesch B, Hanabus
H, Oldenburg J, et al. Long-term recombinant coagulation factor IX
albumin fusion protein (rIX-FP) in hemophilia B: results of a phase 3

34. Saepmi Z, Igawa T, Soeda T, Okuyama-Nishiya Y, Moriyama C,
Wakabayashi T, et al. Identification and multidimensional optimization of an
asymmetric bispecific IgG antibody mimicking the function of factor

Anti-factor IXa/X bispecific antibody ACE910 prevents joint bleeds in
a long-term primate model of acquired hemophilia A. Blood 2014;
124: 3165-3171.

36. Uchida M, Sambe T, Yoneyama K, Fukazawa N, Kawamishi T, Kobayashi
S, et al. A first-in-human phase 1 study of ACE910, a novel factor VIII-
imetic bispecific antibody, in healthy subjects. Blood. 2016; 127:
1633-1641.

37. Shima M, Hanbusa H, Taki M, Matsushita T, Sato T, Fukutake K,
et al. Factor VIII-Mimetic Function of Humanized Bispecific Antibody in

therapeutic targeting anti-thrombin to rebalance the coagulation
system and promote hemostasis in hemophilia. Nat Med. 2015; 21:
492-497.

A subcutaneously administered investigational RNAi therapeutic, Fitusiran (ALN-AT3), targeting Antithrombin for treatment of
hemophilia: interim results in patients with hemophilia A and B.

40. Nordfang O, Valentin S, Beck TC, Hedner U. Inhibition of extrinsic
pathway inhibitor shortens the coagulation time of normal plasma

41. Hilden I, Lauritzen B, Sørensen BB, Clausen JT, Jespersgaard C, Krogh
BØ, et al. Hemostatic effect of a monoclonal antibody mAb21
2021 blocking the interaction between FXa and TFPI in a rabbit hemophilia

42. Agero H, Ovgaard RV, Petersen MB, Hansen L, Hermit MB, Sørensen
MH, et al. Pharmacokinetics of an anti-TFPI monoclonal antibody
(conzumab) blocking the TFPI interaction with the activte site of FXa
in Cynomolgus monkeys after iv and sc administration. Eur J Pharm


