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Review Article

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

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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 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.

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ABBREVIATIONS

AAV: Associated Adenovirus; ABR: Annualized Bleeding Rate; aPTT: activated Partial Thromboplstin 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: Monoclonal antibodies; N9-GP: Novo Nine glycopeghylated; PD: Pharmacodynamics; pdCFCs; plasma derived Clotting Factor Concentrates; pdFIX: plasma derived FIX; PEG: PolyEthylen Glycol; PFM: protein Free Material; PK: Pharmacokinetics; PTPs: 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

new cases of HIV infection and after 1993, when also the anti-HCV screening of blood donors became mandatory, none new cases of hepatitis have been observed worldwide among hemophilia patients. Although some concerns exit so far about the non-lipid enveloped viruses, like Parvovirus B19 [2] and Parvo4 [3], the currently available pdCFCs are safe as ever before. On the other hand, virus safety issue is still worrying the hemophilia treaters and patients [4,5] because the memory of epidemic is still deeply imprinted in their minds.

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 lyophilized 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 1990and 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 or truncated (Table 1). FVIII Single Chain produced by CSL Behring is a new BD deleted concentrates, being the heavy chain and the light chain fused by a covalent link [9]. A new BDD rFVIII concentrates was co-expressed together with Fc (rFVIIIFc) 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 rFVIIIFca large cohort of severe PTPs (n=118) underwent individualized prophylaxis 25-65 IU/kg every 3-5 days rFVIIIFc (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 KDa PEGylated 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 (Nonacog betapegol) 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,

Table 1: Summary of characteristics and PK outcomes of new EHL rFVIII concentrates.

Trade Name	Compound	Company	Structure	Half life (hrs)	Clearance (mL/h/kg)	Vd (mL/kg)	Ref.
Adynovate® (BAX855)	Octocog alfa pegol	Baxalta	Full length rFVIII pegylated	10.5-18.13	0.73-4.79	---	20
BAY94-9027	Damoctocog alfa pegol	Bayer	BDD rFVIII, pegylated	13.7-28.1	0.90-2.00	35-50	11
Eloctate®	Efraloctocog alfa	Biogen/SOBI	BDD FVIII, Fc fusion	14.3-24.5*	1.31-3.29*	39.3-68.3*	10
NovoEight-GP®	Turoctocog alfa pegol	NovoNordisk	BDD truncated FVIII, pegylated	11.79	1.05-2.90	29.6-52.2	12
Nuwiq®	Simoctocog alfa	Octapharma	BDD FVIII	10.49-15.67	3.13-4.97	50.1-82.1	14
rVIII-SingleChain	Ionoctocog alfa	CSL Behring	BDD FVIII, light and heavy chains linked	14.5	2.64	50.0	24

*Extreme values of PK 25IU/kg and 65 IU/kg
Abbreviations: rFVIII: recombinant FVIII; BDD: B Domain Deleted; Vd: Volume of Distribution

Table 2: Summary of characteristics and PK outcomes of new EHL rFIX concentrates.

Name	Compound	Company	Structure	Half life (hrs)	Clearance (mL/h/kg)	Vd (mL/kg)	Ref.
Alprolix®	Eftrenonacog alfa	Biogen/SOBI	rFIX, Fc fusion	56.5-57.6	2.84-3.44	183-262	15
				71.4-94.5	2.80-3,60	277-356	29
Idelvion®	Albutrepenonacog alfa	CSL-Behring	rFIX, albumin fusion	91.57-104.71	0.73-0.87	85.4-119.4	26
NovoNine GP®	Nonacog beta pegol	Novo Nordisk	rFIX, pegylation	82-110	0.65-0.76	90-101	27

Abbreviations: rFIX: Recombinant FIX; Fc: Fragment Crystallizable; Vd: Volume of Distribution

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 nonacog beta pegol and albutrepenonacog alfa resulted about 5 times longer than that of nonacog alfa and eftrenonacog alfa half-life 2.4 time longer (Table 3). These impressive data are partially biased by the different sampling design of PK adopted in the two studies, shorter for pdFIX and nonacog alfa [26,27]. In the eftrenonacog alfa study [29]the sampling times of nonacog alfa was prolonged up to 72 hrs and its half-life resulted quite long, as also in other PK studies using the same sampling design [30,31]. This is the reason why the ratio eftrenonacog alfa/nonacog alfa resulted 2.43 about the half of other ratios (4.79 and 5.31 for nonacog alfa pegol and albutrepenonacog alfa, respectively) (Table 3). The clearance of eftrenonacog alfa resulted quite high (3.2 ml/h/kg) and the Volume of distribution very large (277.8-356.8 ml/kg; normal plasma volume 40-41 ml/kg) [29] (Table 3), probably due to the uptake by cells bearing FcRn. Consequently, the ratios of clearance of eftrenonacog alfato nanocog alfa resulted smaller than those of other EHL rFIX (Table 3).

Efficacy of new EHL CFC has been evaluated in phase III study whose outcomes were very good. ABR during prophylaxis studies has been compared with previous on demand periods. In the nonacog alfa Paradigm 2 study, [32] the ABR in the on demand group (15 patients) was 15.6 and in the weekly prophylaxis 10 IU/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 was 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 and29 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 anticoagulantones, 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.

	Product	Clearance (mean)	Clearance ratios pdFIX/ or rFIX/EHL FIX		Half-life (mean)	Half-life ratios EHL FIX/ or EHL FIX/rFIX	Ref.
		mL/h/Kg			Hours		
Nonacog alfa pegol	N9-GP	0.71			92.67		27
pdFIX	pdFIX	5.48	pdFIX/N9-GP	7.7	17.79	5.21	
Nanocog alfa	rFIX	6.99	rFIX/N9GP	9.8	19.34	4.79	
Eftrenonacog alfa	rFIX-Fc	3.20			82.9		29
Nanocog alfa	rFIX	6.30	rFIXFc	2.0	34.15	2.43	
Albutrepenonacog alfa	rIX-FP	0.75			91.57		26
pdFIX	pdFIX	4.76	pdFIX/rIX-FP	6.3	14.59	6.28	
Nanocog alfa	rFIX	5.24	rFIX/rIX-FP	7.0	17.23	5.31	

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.

Name	Compound	Company	Structure	Indication	Stage of development	Mechanism of action
ACE 910	Emicizumab	Chugai/Hoffmann-La Roche	Asymmetric bispecific IgG	Haemophilia A	Phase I/II	ACE910 mimics FVIII cofactor
ALN-AT3	Fitusiran	Alnylam	siRNA	Haemophilia A&B	Phase I	Inhibition of AT
NN- 7415	Concizumab	Novo Nordisk	Humanized monoclonal Ig4	Haemophilia A&B	Phase I	Inhibition of TFPI

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 outcomes on the efficacy of ACE910 derived from a phase III study: in three groups of hemophilia patients treated with ACE910 03, 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 was 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.

New EHL rFVIII concentrates	Half-life (Hours)
N8-GP	19.0
BAY94-9027	19.0
rFVIIIFc	18.8
BAX 855	16.0
FVIII Single Chain	14.0
New EHL rFIX concentrates	**Half-life (Hours)**
N9-GP	92.67
rIXFP	91.57
rFIXFc	77.0

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 transferred 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 insertional 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 regimens. 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. UKHCDO 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 TFPI and AT, seems to be able to rebalance the hemostatic 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 for sure not self for patients and the risk of developing specific antibodies could be high. No antibodies neutralizing the drugs' effect have been detected in the phase I/II of both Mabs. Non-neutralizing antibodies may escape the assay because bound to the drug, creating circulating immune-complexes that may injure tissues by their deposition. In conclusion, these years are full of interesting and outstanding news for hemophilia treatment. The role pf PK and PD is becoming more and more important to tailor the therapy of each patient in order to achieve the best efficacy. Also cost/effectiveness ratio must be kept in mind when switching patients from old to new, presumably more expensive, concentrates. Long term pharmacovigilance will provide us with the real immunogenicity and toxicity of the incoming new drugs. As far as gene therapy for hemophilia is concerned, we are waiting since more than 20 years some good news. Outstanding successes have been achieved in gene transfer but the severe side effects observed (insertional mutagenesis, hepatotoxicity) and the not lifelong or poor expression of deficient factor make this therapy still a dream of the future. On the other hand, none of the more than thousand gene therapy trials for curing the inherited disease or cancer achieved so far the marketing approval. As K.A. High wrote few years the road for gene therapy of hemophilia is long and winding [66].

CONFLICT OF INTEREST

Massimo Morfini acted as paid consultant to Baxter, Novo Nordisk, Pfizer, Kedrion and received a fee as invited speaker at CSL Behring, Kedrion, Novo Nordisk and Octapharma Symposia.

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