Review: Clinical Trial Outcomes

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CLINICAL INVESTIGATION

Clinical efficacy and safety of moroctocog alfa

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The mainstay of treatment for patients with hemophilia A is replacement with either plasma-derived or recombinant (r) coagulation factor (F)VIII. As compared with whole plasma, plasma-derived FVIII concentrates clearly improved the clinical outcome for patients with hemophilia A; however, their use was associated with the transmission of blood-borne viruses such as hepatitis B and C virus and HIV, and prompted the introduction into clinical use of biotechnologically engineered recombinant clotting factor concentrates, with a much lower theoretical risk of transfusion-associated infections. Continued efforts to further improve safety have led to the introduction of newer generations of rFVIII, with each subsequent generation representing an incremental step with regard to pathogen safety. Between 1980 and 1985, research demonstrated that removing the middle portion (the B-domain) of wild-type FVIII protein had no detrimental effect on its procoagulant activity as assessed *in vitro*. This finding was the basis for the development of a recombinant B-domain-deleted FVIII (BDDrFVIII), moroctocog alfa (ReFacto®). Moroctocog alfa albumin-free cell culture (ReFacto AF® in Europe, Xyntha® in the USA) has been developed as a successor to ReFacto. The aim of this article is to describe the clinical evidence about moroctocog alfa and moroctocog alfa albumin-free cell culture.

Keywords: B-domain-deleted factor VIII • factor VIII concentrates • hemophilia

Hemophilia A is a genetic disease, resulting in a deficiency of coagulation factor (F)VIII. It occurs in approximately one out of 5–10,000 male live births [1,2]. The mainstay of treatment for patients with hemophilia A is replacement therapy with either plasma-derived (pd) or recombinant (r) FVIII concentrates [3], which were shown to clearly improve the clinical outcome for patients with hemophilia A until the mid 1980s [4–7]. Until then, the concentrates were produced exclusively from human plasma (Figure 1), without adoption of effective measures to prevent transmission of blood-borne pathogens [3], so that their use was associated with the transmission of blood-borne viruses, such as hepatitis B and C beginning in the early 1980s, and HIV [8]. Although after 1985 the advances in the pd concentrate production process have resulted in improved safety, and no new case of infection has been reported, highly thermoresistant viruses, prions (associated with variant Creutzfeld-Jacob Disease) or new pathogens might still be on a theoretical plan transmitted by this class of products [4,6,9–11].

The clinical introduction of biotechnologically engineered recombinant clotting factor concentrates has virtually eliminated the risk of transfusion-associated infections. This notwithstanding, since the cell line producing the protein is susceptible to infections, continued efforts to improve safety have led to the introduction of newer generations of rFVIII, with each subsequent generation representing an incremental step with regard to pathogen safety. The first generation of rFVIII products contained human serum albumin as a stabilizer in the final formulation [12]. Albumin has been eliminated from the vials of the

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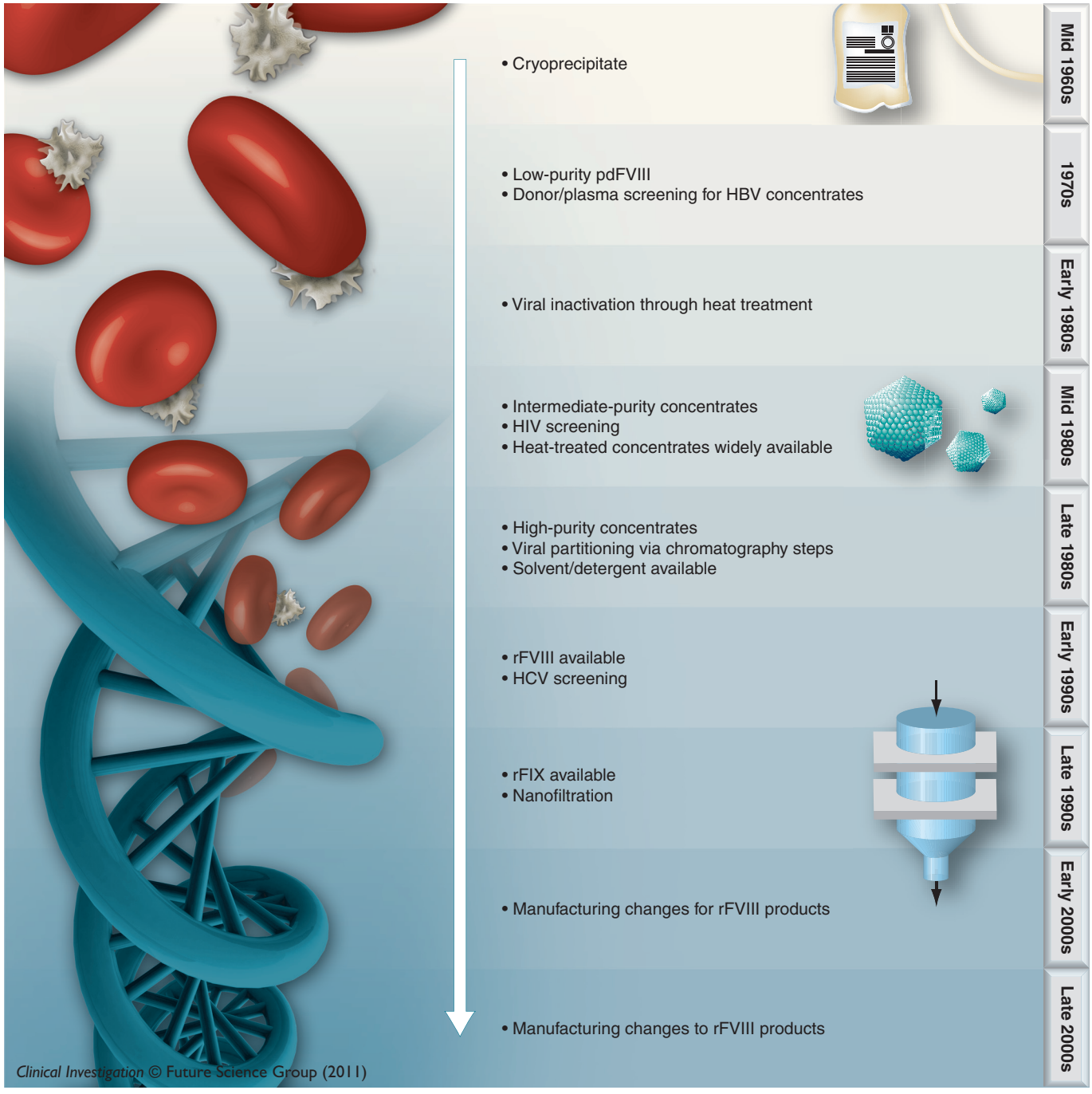

Figure 1. Major steps in the progress of hemophilia treatment.

second-generation products, but human and/or animal protein components were still used in the cell culture processes [13,14]. In third-generation products, all exogenous human- and animal-derived proteins have been removed from the whole process, with the exception of murine monoclonal antibodies, which continued to be used for immunoaffinity purification of rFVIII [15].

B-domain-deleted rFVIII

■ From the laboratory to the clinic

Between 1980 and 1985, research demonstrated that removing the middle portion (the B-domain) of a pdFVIII protein had no negative effect on its activity as assessed *in vitro* [16]. *In vivo* testing in hemophilic dogs demonstrated that pharmacokinetic parameters remained essentially unchanged when compared with the

full-length protein [17]. This finding was the basis for the development of a recombinant B-domain-deleted FVIII (BDDrFVIII), named moroctocog alfa (ReFacto®). The recombinant molecule lacks all but 14 amino acids of the B-domain. The active component in moroctocog alfa is synthesized by a genetically engineered CHO cell line. To create the production cell line, the *BDDrFVIII* gene, encoding the 170-kDa moroctocog alfa primary translation product, was cloned into a mammalian cell expression vector containing a strong viral enhancer and promoter, and transcriptional termination and polyadenylation sequences derived from the *human tissue-type plasminogen activator* gene [18]. In addition, a second vector was designed to express the mouse *DHFR* gene, which was used as a selectable, amplifiable marker gene. These two expression vectors were cotransfected into the *DHFR*-deficient CHO cell line. After selection in nucleoside-free medium, transformants were subjected to methotrexate-induced gene amplification, resulting in amplification of the mouse *DHFR* and *BDDrFVIII* genes. The engineered cell line was cultured in a human serum albumin-rich medium to produce the active molecule. This was subsequently purified from the supernatant by chromatography utilizing a mouse monoclonal antibody and stabilized in the final formulation with sucrose, thereby qualifying ReFacto as a second-generation rFVIII since the beginning. Moroctocog alfa albumin-free cell culture (AF-CC) has been developed as a successor to ReFacto by eliminating albumin from the cell culture medium. Furthermore, in its manufacturing process, the synthetic peptide affinity ligand TN8.2 replaces the murine monoclonal antibody used for the affinity chromatography step for moroctocog alfa and all other currently available rFVIII concentrates, and a 35 nm micropore nanofiltration is added as a virus removal step, expanding upon the viral-safety program currently in use for moroctocog alfa (Figure 2) [19]. Moroctocog alfa (AF-CC) is available for clinical use employing two different potency assignment processes. One has been developed for the USA, Canada and other FDA-based regions of the world, with potency assignment aligned to the one-stage FVIII activity assay. This product is identified by the trade name Xyntha®. The other, identified as ReFacto AF®, has been developed for the EU and the other EMA-dependent regions of the world in agreement with the European Pharmacopoeia, with potency assignment aligned to the chromogenic substrate FVIII activity assay.

■ Moroctocog alfa

Pharmacokinetics

During the licensing process of moroctocog alfa in 1999, a set of studies demonstrated the efficacy, safety and pharmacokinetic characteristics of moroctocog alfa [20–22]. The two studies by Courter and Bedrosian were mainly focused on the clinical evaluation, while Kessler *et al.*, in a three-way crossover design study, compared the pharmacokinetic parameters of two moroctocog alfa formulations (one reconstituted with 5 ml of sterile water, the other reconstituted with 4 ml sodium chloride 0.9%) with those of a pd, full length FVIII preparation (Hemofil M; Baxter, IL, USA), in patients with hemophilia A, to determine bioequivalence of the two products [20]. A total of 18 severe hemophilia A patients with a mean age of 26 years completed the study. Results from this study demonstrated bioequivalence of the two moroctocog alfa formulations, which were found to be bioequivalent to Hemofil M. These three studies were then included in a meta-analysis by Gruppo *et al.* [23], suggesting an inferior pharmacokinetic profile for moroctocog alfa in comparison with full-length products. Subsequently, data inclusion criteria and statistical methods employed in this meta-analysis were widely debated. Different authors pointed out the heterogeneity of the extracted data [24,25], the opportunity choice of the outcome of interest [26], and the comparability of pharmacokinetic results based on one-stage assay methodology [24]. Mikaelsson [27] and Brown *et al.* [25] noted that the meta-analysis included both hemophilia A and B in the full-length group. Van der Bom *et al.* [26] noted that the analysis by Gruppo *et al.* had as a principal outcome the mean yearly number of bleeds and included 13 observational studies (three for moroctocog alfa and 10 for full-length FVIII). Two of three moroctocog alfa studies for a total of 170 out of 245 patients and four of ten full-length studies for a total of 85 out of 324 patients reported mean annual bleeds. For the other studies, the authors 'adjusted' the reported median values to mean values by using a conversion factor of 2.6. Van der Bom *et al.* suggested that this factor is rather high and thus the conclusions of the meta-analysis were possibly unreliable [26].

Finally Di Paola *et al.* compared the pharmacokinetic characteristics of moroctocog alfa with that of full length rFVIII (FLvFVIII; octocog alfa; Advate; Baxter) [28]. Eighteen severe hemophilia A patients aged 18–72 years were enrolled, 17 of which were included in the pharmacokinetic analysis. Results from the pharmacokinetic evaluation of the two products, using the chromogenic substrate assay, demonstrated that moroctocog alfa and octocog alfa are bioequivalent (Table 1).

Laboratory assessment

After moroctocog alfa marketing, it quickly became evident that the one-stage clotting assay underestimates by up to 40% the plasma levels of FVIII after the infusion of moroctocog alfa in patients with hemophilia [29]. A deeper insight into the recovery discrepancy found between moroctocog alfa and wild-type FVIII came

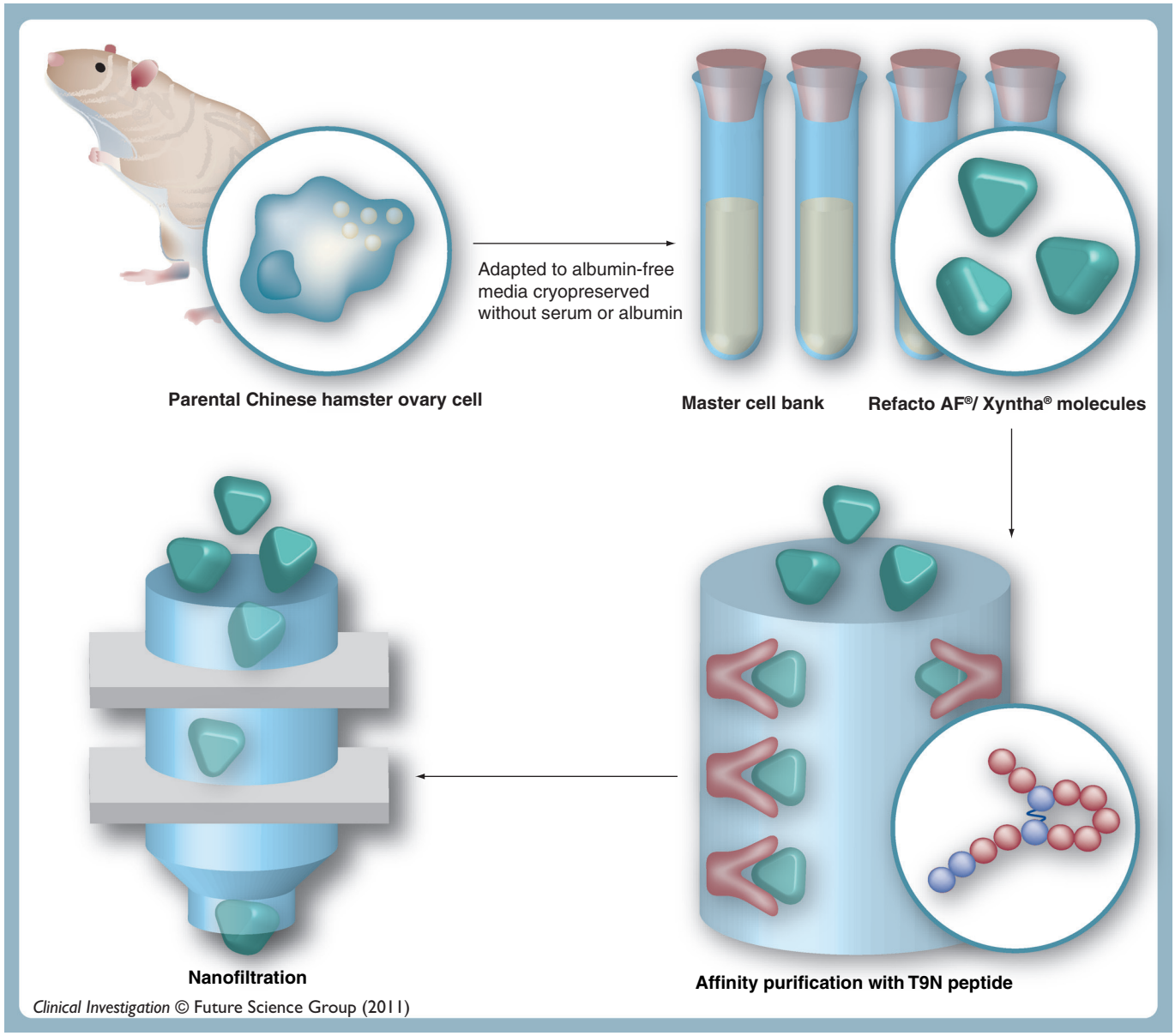

Figure 2. New features in the production process of moroctocog alfa albumin-free cell culture.

with the application of different assay methods, particularly by comparison of the one-stage clotting assay with the chromogenic method [30]. For this reason a moroctocog alfa reference standard (ReFacto laboratory standard [RLS]), aiming to minimize the aforementioned underestimation, was introduced and found to be useful in clinical monitoring of the postinfusional level of moroctocog alfa [30,31]. RLS was subsequently updated for all reformulations or modifications of moroctocog alfa AF-CC. Santoro *et al.* compared FVIII activity levels, measured with the chromogenic assay, versus one-stage assay after ReFacto reformulation in 2005 [32]. The authors found the one-stage method to be more sensitive to lower plasma concentrations of FVIII while the measured maximum plasma concentration (C_{max}) was slightly higher than theoretical values and independent of the assay used. C_{max}, area under the curve and volume of distribution at steady state presented nonsignificant differences among the methods and standards used, so that the authors concluded that safe and effective monitoring of moroctocog alfa in clinical use by the one-stage assay was possible [32].

■ ReFacto AF/Xyntha (moroctocog alfa AF-CC)

Pharmacokinetics

The pharmacokinetics of moroctocog alfa AF-CC were characterized in a single-dose, randomized, double-blind, two-period crossover study by Giel *et al.*,

comparing it with moroctocog alfa [33]. In all, 30 previously treated patients (PTPs) with severe hemophilia A received a single dose of moroctocog alfa 50 IU/kg followed by the same dose of moroctocog alfa AF-CC; a validated chromogenic substrate assay procedure was used to determine FVIII:C. A total of 25 out of 30 enrolled patients were evaluable for pharmacokinetic analyses. Plasma concentrations of FVIII increased sharply in response to the 2-min intravenous infusion of both molecules, with a peak concentration of 1.16 ± 0.17 and 1.15 ± 0.16 IU/ml for moroctocog alfa AF-CC and moroctocog alfa, respectively. After the end of the infusion, the decline of the plasma concentration of FVIII exhibited multiphasic disposition characteristics. In the initial phase, plasma concentrations dropped at a rate consistent with a relatively rapid but limited distribution into an extravascular space. The steady-state volume of distribution was 49.9 ml/kg for moroctocog alfa AF-CC and 51.1 ml/kg for moroctocog alfa. During the terminal phase, the rate of decline in plasma concentrations was slower, with a half-life of approximately 9.9 ± 3.2 h for moroctocog alfa AF-CC and 10.9 ± 4.5 h for moroctocog alfa (Table 1).

A double-blind randomized pharmacokinetic crossover study by Recht *et al.* found a complete bioequivalence between moroctocog alfa AF-CC and octocog alfa using a central laboratory and one-stage methods [34]. Analysis of pharmacokinetic parameters for moroctocog alfa AF-CC and octocog alfa demonstrated, in 30 subjects, that the two products were pharmacokinetic equivalent: 90% CIs around the ratios of moroctocog alfa AF-CC to octocog alfa geometric least squares means of the key pharmacokinetic parameters (AUCt, AUC_{∞}, K-value) were all within the pharmacokinetic-equivalence window of 80–125% (Table 1). Similar *in vivo* recovery estimates were also found. Baseline moroctocog alfa:AF-CC pharmacokinetic parameters were also estimated based on the manufacturer's labelled potency. Moreover, the pharmacokinetic parameters after moroctocog alfa AF-CC remained unchanged with repeated use over time in 25 subjects assessed 6 months later: 90% CIs about the ratios of moroctocog alfa AF-CC month 6-to-baseline geometric least squares means of key parameters were all within the 80–125% equivalence window. These results were confirmed by Widynga *et al.* [35] who observed *in vivo* recovery values of 101 ± 20% (mean ± SD) in a series of patients undergoing surgery. For patients undergoing full pharmacokinetic assessments while on continuous infusion, the mean plasma FVIII:C versus time profile increased sharply after the start of intravenous infusion of moroctocog alfa:AF-CC. After the end of the infusion, the decline of FVIII:C exhibited biphasic disposition with an elimination phase $t_{½}$ of 16.7 ± 5.4 h (mean ± SD).

Laboratory assessment

The rationale for developing two different brands, Xyntha and ReFacto AF, was based on the manufacturer objective to align the potency assignment of moroctocog alfa AF-CC with the one-stage assay more commonly used in clinical monitoring. This was actually possible only in the FDA-dependent regions of the world, since EMA continued to ask for titration of the drug based on a chromogenic substrate-based test. Consequently, Xyntha was developed following the FDA requirement, and the trial by Recht *et al.* found that the moroctocog alfa AF-CC has pharmacokinetic equivalence to octocog alfa using either a

Table 1. Comparative pharmacokinetic evaluation.

Ratio of least squared means and 90% CI	C_{max}	AUCt	AUC_{∞}	K values	$t_{1/2}$	*In vivo* recovery	Ref.
BDDrFVIII vs Hemofil M	102 (98–106)	115 (110–119)	118 (113–124)	103 (99–107)	109 (105–116)	103 (99–107)	[20]
BDDrFVIII-A† vs Hemofil M	105 (101–109)	114 (108–119)	116 (110–122)	106 (102–110)	104 (98–115)	107 (103–111)	[20]
BDDrFVIII-A vs BDDrFVIII B‡	97 (94–101)	101 (97–104)	102 (99–105)	104 (96–112)	104 (96–112)	97 (94–101)	[20]
BDDrFVIII vs BDDrFVIII AF	ND	99.5 (93.8–105.6)	98.1 (92.2–104.3)	100.6 (97.6–103.6)	ND	100.8 (98.3–103.6)	[33]
BDDrFVIII vs Advate	88–104.3	95.4–115.2	95.3–112.8	88.0–104.3	ND	ND	[28]
BDDrFVIII AF vs Advate	ND	83.3–96.9	81.6–94.8	92.5–108	ND	ND	[34]

†BDDrFVIII-A: BDDrFVIII recostituited with 5 ml sterile water.

‡BDDrFVIII-B: BDDrFVIII recostituited with 4 ml sodium chloride 0.9%.

ND: Not determined.

single centralized one-stage method for FVIII:C or with the assay used for titration of drug potency used also to test the plasma FVIII:C concentration in patient plasma [34]. To adhere to EMA requirements, ReFacto AF was developed for Europe, and European laboratories still have the option to use the RLS, even if the results by Santoro *et al.* [32] might also be applied to ReFacto AF making the use of the laboratory standard redundant. It has to be finally noted that the only difference between Xyntha and ReFacto AF is in the procedure used to label their potency (i.e., in the amount of active substance in the vial, being the active molecule identical in the two brands).

Clinical evidence

■ ReFacto (moroctocog alfa)

Two Phase III long-term studies were conducted to evaluate safety and efficacy of moroctocog alfa in patients with hemophilia A. The studies were performed in PTPs [22] and in previously untreated patients (PUPs) [21].

During the Phase III long-term study in PTPs by Courter *et al.*, 113 patients received a total of 47,674 moroctocog alfa infusions, ranging from 4 to 1769 infusions/patient (median 327) for a cumulative dose of 101,431,407 IU [22]. A total of 10,882 bleeding episodes were treated during the study using a mean dose of 31 IU/kg. Of the bleeding episodes, 73% were treated with one infusion, 15% with two infusions, 6% with three infusions and 6% with four or more infusions. Approximately 95% of the infusions (17,040/17,979) were assessed for hemostatic efficacy; overall, the patient or the investigator rated 92% (15,744/17,040) of the infusions as providing an excellent or good response.

A total of 85 patients received prophylaxis during the study. Overall, the patients receiving moroctocog alfa as prophylaxis experienced a mean of ten breakthrough bleeding episodes (median: seven; range: 0–42) per year using an average dose of 27 IU/kg. This represented a twofold reduction in the bleeding frequency compared with their on-demand treatment periods (mean: 25 episodes per year [median: 20]; range: 0–35). These results are consistent with efficacy data from other trials using rFVIII products, particularly considering the age of the patients (median age: 26 years). During the prophylactic period, 12% of patients experienced no bleeding episodes and 17% of the patients had no on-demand treatment for breakthrough bleedings. One of the 113 (0.8%) patients in this study, a 53-year-old Caucasian man, developed an inhibitor.

Similar results (other than the percentage of developing inhibitors) were found in the PUP trial by published by Courter *et al.* [21]. In particular, a total of 32,442 infusions were administered to 101 PUPs over 30,893 exposure days (ED) for a total of 36,311,472 IU. The median dose per patient was 169,700 IU (range: 500–2,794,500 IU). The median number of infusions per patient was 218 (range: 1–1476). Of the 2715 bleeding episodes that were treated, 66% (1794/2715) were resolved with one infusion, 85% (2296/2715) were resolved with one to two infusions and 93% (2525/2715) were resolved with one to three infusions. Of the 2375 infusions rated by the investigators, 93% (2215/2375) were rated as providing an 'excellent' or 'good' response. A total of 27 patients received routine prophylactic treatment, which significantly reduced breakthrough bleeding episodes twofold when compared with patients who were treated on-demand. The mean dose was 56 IU/kg for routine primary prophylaxis and 53 IU/kg for on-demand therapy for bleeding episodes in patients who were assessed to be inhibitor-free at the time of infusion; 32% of patients developed inhibitors. Of these, 16 patients were high responders (peak titer ≥5 Bethesda units [BU]). A total of 42 patients in both the PUP and PTP studies was operated on and the overall efficacy of moroctocog alfa was rated as excellent or good for 99.6% of the infusions [36].

A retrospective study by Steltjes *et al.* [37] evaluated the hemostatic effect of continuous infusion of moroctocog alfa in hemophilia patients undergoing surgery and requiring more than five consecutive days of treatment. The outcomes were estimated blood loss during and after surgery (including number of red blood cell units transfused), total usage of moroctocog alfa, infusion rate and use of additional bolus injections of FVIII products. In all, 16 patients from eight centers underwent a total of 20 procedures. Hemostatic outcome was assessed as excellent or good in 75% of procedures. In the remaining cases, where efficacy was rated as moderate, patients had undergone surgical procedures known to have a high risk of bleeding (removal or replacement of a prosthesis, total knee replacement, recurrent procedures on the same joint). In those cases where blood loss did occur during surgery, the amount of loss was generally within the normal range for nonhemophilic patients for the type of surgery performed.

Two postmarketing surveillance studies confirmed the original data published by Courter *et al.* [38,39]. In total, 60 patients, 58 PTPs and two PUPs, were enrolled in an open-label, multicenter, postmarketing surveillance study by Smith *et al.* [38]. Surgical prophylaxis was evaluated in seven patients who underwent elective surgery. A total of 32 patients aged <1–66 years (median: 19.5) received prophylaxis and 28 patients, aged 1–71 years (median: 33.5), received on-demand treatment. The majority of patients had

severe hemophilia A (FVIII:C <2%): 84.4% in the prophylaxis group and 85.7% in the on-demand group. Prophylaxis with moroctocog alfa was associated with a median of 6.7 bleeds per year (range: 0–37). The assessment of the final outcome in prophylactic treatment was judged to be excellent or effective by the investigators in 93.1% of patients. Moroctocog alfa resolved 92.8% of bleeds with one or two infusions. The investigators' assessment was excellent or good for 98.2% of bleeds treated with moroctocog alfa. Hemostasis was achieved in all seven surgical cases and moroctocog alfa resulted in an excellent or good response for each. One PTP developed a high-titer inhibitor (peak 75 BU) and one minimally treated patient developed a low-titer inhibitor. One PUP developed a transient low-titer inhibitor (0.4 BU).

In another postmarketing surveillance study by Pollmann *et al.*, 217 patients from 38 hemophilia centers were enrolled [39]. Of these patients 81% had more than 100 EDs and 12.9% 0–50 EDs. A total of 153 of 217 patients adequately completed diary information and were thus analyzed for the primary outcome; they experienced a median of seven (interquartile range 1.4–18.6) bleeding episodes/year. Data concerning the number of patients on prophylaxis or on-demand treatment were not provided by the authors, who reported that patients treated on prophylaxis experienced a median of 4.4 (1.1–9.3) bleeds/year, while patients treated on-demand experienced a median of 22.8 (11.3–29.0) bleeds/year. Overall, most physicians (41/43, 95.3%) were 'very satisfied' or 'satisfied' with the efficacy of moroctocog alfa in the treatment of bleeding episodes. Overall, six patients (2.8%) developed *de novo* inhibitors, three of which were high titer. Four of these patients were at high risk (0–50 ED) for inhibitor formation, one was at intermediate risk (51–100 ED) and one was at low risk (>100 ED).

The risk of inhibitor development was also investigated by Gringeri *et al.*, who found the same rate of inhibitors observed with other FVIII products [40]. The trial analyzed a prospective cohort of 25 severe PTPs exposed to FVIII products other than moroctocog alfa for more than 50 EDs and then having received more than one infusion of moroctocog alfa; and the retrospective cohort of all Italian patients who switched to moroctocog alfa (94 patients). The retrospective cohort was subdivided on the base of the number of EDs: one group with less than 50 EDs (high risk of inhibitor development: 19 patients) and the second group with more than 50 EDs (low risk of inhibitor development: 75 patients). Of 25 prospective PTPs enrolled, 24 did not develop an inhibitor when switched from pdFVIII or octocog alfa to moroctocog alfa and one patient developed a high titer inhibitor (4%). The inhibitor titer was initially low (2 BU) but reached a peak value of 30 BU in 2 months. On the whole, in the entire retrospective Italian cohort, no inhibitor was found in low-risk PTPs, whereas one of the 19 PTPs (5%) still at high risk of inhibitor development became inhibitor positive. This severe hemophilia patient had 13 EDs (ten of which were with moroctocog alfa), the peak titer was 15 BU and 17 months later, when the inhibitor titer decreased to 1 BU, he started immunotolerance with moroctocog alfa and the inhibitor disappeared 20 months later.

■ ReFacto AF/Xyntha (moroctocog alfa AF-CC)

The clinical segment of the study by Recht *et al.* was an open-label, prospective multicenter trial to assess safety and efficacy over time [34]. Patients received a defined prophylaxis regimen of moroctocog alfa AF-CC for a minimum of 50 ED over 6 months. The prophylaxis treatment regimen was consistent with current literature [41,42] and was initiated at dose of 30 IU/kg given three-times weekly. Predefined 'escape' criteria in response to bleeding episodes allowed for prophylaxis dose escalation to higher intensity regimens, initially to 45 ± IU/kg three-times weekly and then to more frequent administration or higher doses as determined by the investigator. In addition to routine prophylaxis, intermittent prophylaxis in the form of additional infusions of the study drug was allowed if it was believed such treatment was required to prevent bleeding for an upcoming activity or procedure. All bleeding episodes were treated at the discretion of the investigator, mostly by the patient himself in the homecare setting based on investigator guidance ('on-demand' treatment). Moroctocog alfa AF-CC was to be exclusively used either on prophylaxis to prevent bleeding or on-demand for treatment of bleeding, whether spontaneous or traumatic. All patients enrolled in the study were male, with a median age of 24 years (mean: 27.7 years; range: 12–60 years). Most of the patients (80.9%) were 17 years of age or older and white (94.7%). A total of 94 patients received treatment with moroctocog alfa AF-CC in study 1. The median number of ED per patient was 76 (range: 1–92), and the median dose per infusion was 30.2 IU/kg (range: 6.4–76.9). Two out of 94 (2.1%) patients developed transient, low-titer inhibitors, which means an inhibitor rate below the predefined FDA acceptable value of 4.4%. Alternatively described, there is a 95% probability that the true population rate of inhibitor development with use of moroctocog alfa AF-CC is below 4.07%. The cut off value for the inhibitor rate to be considered acceptable in the study was determined by Bayesian estimation, assuming as prior distribution the inhibitor

rate probability assessed in all the patient series previously treated with moroctocog alfa. This innovative approach, proposed to the FDA and subsequently adopted as the golden standard to assess immunogenicity in PTP patients, has the advantage of building more reliable variability limits around the mean value ascertained in a single study and would be the only one to keep all the currently brands on the market if reassessed as of today (most brands were marketed before FDA started to require a predefined rate of inhibitor development) [43,44]. All 94 patients on study 1 received moroctocog alfa AF-CC for prophylaxis; the median dose administered was 30 IU/kg (range: 6.8–76.9). Only seven prophylaxis dose escalations were prescribed in six patients. Most patients (57/94; 60.6%) reported no spontaneous bleeding episodes while on routine prophylaxis, while almost half of patients (43/94; 45.7%) reported no bleeding at all (spontaneous or traumatic) while on routine prophylaxis. A total of 53 out of 94 patients also received moroctocog alfa AF-CC as on-demand treatment for breakthrough bleeding and surgical prophylaxis among others; the median dose administered was 30 IU/kg (range: 6.4–74.4). The majority of bleeding episodes (173/187; 92.5%) resolved with one or two infusions. Similar results were found in the study 2. In all, 110 patients were enrolled in study 2; 104 of these on prophylaxis regimens and six on the on-demand regimen. Moroctocog alfa AF-CC was effective in prevention and treatment of hemorrhages: 24% of these patients had no hemorrhages and 49% had no spontaneous hemorrhages during routine prophylaxis [34].

Windyga *et al.* enrolled patients with severe or moderately severe (FVIII:C < 2%) hemophilia A scheduled to undergo elective major surgery [35]. The patients should have a requirement for postoperative FVIII replacement therapy over a period of at least six consecutive days. A total of 25 patients received moroctocog alfa AF-CC by bolus injection or by continuous infusion at the investigators' discretion. At baseline (~4 weeks before surgery), following a 3-day washout after the previous FVIII administration, a 50 IU/kg infusion of moroctocog alfa was administered as intranvenous bolus for recovery or as continuous infusion for clearance rate individual assessment, respectively. Investigators reported their target FVIII activity level for surgery and used results from the baseline assessments to plan the initial moroctocog alfa dose and injection frequency or infusion rate. The primary end point was hemostatic efficacy during the

Table 2. Ongoing studies.

Study title	NCT ID	Study design	Included patients	Outcome measures
Study Evaluating Safety and Efficacy of Moroctocog Alfa (AF-CC) in Previously Treated Hemophilia A Patients	NCT00914459	Nonrandomized, open-label trial	PTPs with more than 150 EDs, pediatric	Pharmacokinetics and incremental recovery of moroctocog alfa in pediatric subjects less than 12 years of age after a single exposure to moroctocog alfa AF-CC
Study of Safety and Efficacy of ReFacto AF in Previously Untreated Hemophilia A Patients in the Usual Care Setting	NCT00950170	Nonrandomized, open-label trial	PUPs with less than 6 years old	Proportion of subjects who develop clinically significant FVIII inhibitors. Efficacy end points include annualized bleeding event rate and responses to the first on-demand treatment with moroctocog alfa
Study Evaluating Safety of Patients Switching to ReFacto AF in Usual Care Settings	NCT00884390	Nonrandomized, open-label trial	PTPs with more than 150 EDs	Inhibitor development
Study Evaluating Prophylaxis Treatment and Characterizing Efficacy, Safety, and Pharmacokinetic of B-Domain-Deleted Recombinant FVIII	NCT00543439	Randomized, open-label trial	PTPs with more than 20 EDs	The annualized bleeding event rate in prophylaxis on-demand therapy, and high vs low frequency prophylaxis regimens; pharmacokinetic properties of moroctocog alfa AF-CC at initial exposure and after repeated exposure
Study Evaluating Pharmacovigilance of ReFacto AF	NCT00895037	Prospective observational, open-label trial	All patients	Reporting of adverse events; efficacy in controlling bleeding
Study Evaluating the Safety of Xyntha in Usual Care Settings	NCT00765726	Nonrandomized, open-label trial	PTPs with more than 150 EDs	Percentage of patients with FVIII inhibitor development

Full information of the trials listed in the table can be found by locating the trial record in [101] by using the NCT ID.

ED: Exposure day; PTP: Previously treated patients; PUP: Previously untreated patients.

Table 3. Key studies.

Study (year)	Study design	Included patients (PUPs/PTPs)	Outcome measures	Inhibitor rate	Ref.
Moroctocog alfa					
Courter & Bedrosian (2001)	Open-label, multicenter prospective study	113 PTPs	Bleeding rate, hemostatic efficacy, safety	0.008	[22]
Courter & Bedrosian (2001)	Open-label, multicenter prospective study	101 PUPs	Bleeding rate, hemostatic efficacy, safety	0.3	[21]
Smith *et al.* (2005)	Open-label, multicenter postmarketing survillance study	60 patients (58 PTPs and 2 PUPs)	Bleeding rate, hemostatic efficacy, safety	0.5 PUPs 0.03 PTPs	[38]
Pollmann *et al.* (2007)	Open-label, multicenter postmarketing survillance study	217 patients: (PUPs 16 and PTPs 101)	Bleeding rate, hemostatic efficacy, safety	0.2 PUPs 0.03 PTPs	[39]
Stieltjes *et al.* (2004)	Open,label, retrospective, noncomparative, multicenter study	16 PTPs	Bleeding rate, hemostatic efficacy, safety	0.06	[37]
Gringeri *et al.* (2004)	Two cohorts: Prospective Retrospective multicenter	 Prospective: 25 PTPs Retrospective: 94 patients, 19 of which with less than 50 EDs and 75 with more than 50 EDs	Inhibitor rate	 Prospective: 0.04 Retrospective: 0.05 in patients with <50 EDs and 0 in patients with >50 EDs	[40]
Moroctocog alfa AF-CC					
Recht *et al.* (2009)	Study 1: randomized, double blinded, crossover study to evaluate pharmacokinetic equivalence between moroctocog alfa and octocog alfa Study 2: open label, prospective, multicenter study	204 PTPs	Pharmacokinetic evaluation, bleeding rate, hemostatic efficacy, safety	0.02	[34]
Windyga *et al.* (2010)	Open-label, multicenter prospective study	25 PTPs	Hemostatic efficacy during surgical procedure through 1 h after surgery completion, safety	0.08	[35]

ED: Exposure day; PTP: Previously treated patients; PUP: Previously untreated patients.

surgical procedure until 1 h after surgery completion. A total of 30 patients were enrolled and treated with at least one dose of moroctocog alfa; 25 patients were evaluable for efficacy. Outcomes were overall favorable against a background of multiple major surgical procedures. All hemostatic efficacy ratings were 'excellent' or 'good'. End-of-surgery hemostasis ratings, the primary efficacy end point, were excellent for 72% (18/25) and good for 28% (7/25) of patients. Hemostasis ratings following the initial postoperative period were excellent for 92% (23/25) and good for 8% (2/25) of patients. Intraoperative blood loss was rated as normal in all patients. Of the 13 patients that had postoperative blood loss, ten were rated as normal. A total of ten bleeding episodes in seven patients were reported in the postoperative setting and required on demand treatment with moroctocog alfa. Seven cases were due to injury, three of which occurring to the operated joint, and three were spontaneous. When bolus and continuous infusion mode of administration were separately analyzed, both gave similar results as far as efficacy and safety are concerned.

Ongoing studies

Six ongoing trials were found by searching clinicaltrial.gov [101]. All trials are sponsored by Pfizer. Four of these are in PTPs (one included minimally treated patients with more than 20 EDs), one trial is in PUPs and another is in all types of hemophilia A patients. Except for one trial in PTP pediatric hemophilia A patients (<12 years old, the primary outcome of this trial is pharmacokinetics and incremental recovery of moroctocog alfa AF-CC), all trials focus their primary outcome on efficacy (reduction in bleeding rate) and safety (inhibitor incidence) of treatment. One of these, the only one designed as a randomized clinical trial, measures bleeding rate in prophylaxis versus on demand treatment and in high- versus low-frequency prophylaxis regimens (Table 2).

Conclusion

Both ReFacto and ReFacto AF/Xyntha were extensively studied before and after their appearance on the market (Table 3), and their efficacy and safety were thoroughly demonstrated. When used on demand, the rate of effective treatment was 98% and it was 92% for patients undergoing surgery, while the median annualized bleeding event rate (number of bleeding per patient per year) for patients treated on prophylaxis was 5.5 (4–7). The overall crude inhibitor rate was 47/755 (6%) in all patients, 13/617 (2%) in PTPs and 34/138 (24%) in PUPs.

The amount of evidence available for the two products is huge and, if one considers the two being interchangeable, having been found to be bioequivalent [33], the overall evidence available for the molecule is the largest ever for a recombinant product.

Currently there is no evidence of higher immunogenicity of the moroctocog alfa molecule as compared with the octocog alfa. The *in vitro* and *ex vivo* experiments, which hypothesized higher neo-antigenicity of the molecule [45] were not confirmed by clinical data [21,22,34,35].

Similarly, experimental evidence suggested that moroctocog alfa might undergo an accelerated clearance [46], thus requiring higher dosages of moroctocog alfa as compared with octocog alfa. The report by Rea *et al.* found no evidence of such a difference in the population of UK patients switched from FL to BDD and back to FL [47]. Particularly, There was no significant difference in coagulation factor usage (moroctocog alfa median 4803 IU/kg/year, range 659–11,304; octocog alfa median 5349 IU/kg/year, range 1691–10,146). In this relatively small cohort no evidence was found of inhibitor appearance in PTPs switching between different rFVIII molecules.

In conclusion, considering the pharmacological and manufacturing process characteristics – made unique by the removal as a potential source of contaminating animal-derived protein of the mouse antibody in the purification step – and the excellent clinical record make moroctocog alfa AF-CC a one-step forward product for the treatment and prevention of bleedings in hemophilia A.

Future perspective

It is hard to imagine any subsequent improvement in the development of FVIII concentrates as far as bloodborne infections and viral safety are concerned. Hot issues in attempting to improve our toolbox for hemophilia A treatment are reducing the immunogenicity of the molecule, improving its pharmacokinetic profile, assessing treatment options different from the classical factor concentrate administration. To modulate immunogenicity, we mainly expect to see whether different starting regimens might have a role in inducing tolerance to FVIII, including the possibility to treat fetuses in utero; attempts to engineer the molecule and reduce its antigenicity has so far been unsuccessful.

To improve the pharmacokinetic profile, some prolonged half-life molecules are in advanced preclinical evaluation, and almost all of them are PEGilated BDD-rFVIII; the aim is to provide effective prophylaxis with once a week endovenous infusion, which, particularly if coupled with new infusion devices (prefilled one-shot self-diluting syringes), might allow more patients to stick to prophylaxis.

Exciting new perspectives are represented by: cure of the disease through gene therapy (this approach is currently on hold, due more to ethical than feasibility reasons); and the development of small peptides mimicking activated factor X (these could be available by oral route and effective in the treatment of both hemophilia A and B, with or without inhibitors). Is that a dream? Let us look forward, and we will have an answer, hopefully within no more than 10 years.

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Executive summary

- Subsequent generations of recombinant factor concentrates have progressively increased product safety.
- B-domain-deletion has been introduced to make the protein production and engineering more efficient without any loss of activity.
- B-domain-deleted factor VIII (ReFacto®) has been demonstrated to be effective and safe, and equivalent for clinical efficacy and immunogenicity to both plasma-derived and full-length recombinant factor VIII.
- Previous formulations of ReFacto were deemed to require a specific factor VIII assay for clinical titration. This is not anymore required on a clinical ground with new generation products.
- Xyntha™/ReFacto AF have been introduced as the sole albumin-free, mouse antibody-free, nanofiltered recombinant factor VIII available on the market.
- Xyntha and ReFacto AF differ uniquely for the titration procedure, which is activated partial thromboplastin time based for Xyntha (FDA regions) and chromogenic assay based for ReFacto AF (EMA regions).
- Xyntha/ReFacto AF efficacy and safety has been widely demonstrated for on-demand, prophylaxis and surgical usage. The incident rate of inhibitor has been found in the expected range for recombinant products.

Bibliography

Papers of special note have been highlighted as:

- ■ of interest
- ■■ of considerable interest

1 Hay C. The UK Haemophilia Database: a tool for research, audit and healthcare planning. *Haemophilia* 10(S3), 21 (2004).

2 Iorio A, Oliovecchio E, Morfini M, Mannucci PM. Italian Registry of Haemophilia and Allied Disorders. Objectives, methodology and data analysis. *Haemophilia* 14(3), 444–453 (2008).

3 Mannucci PM, Tuddenham EG. The hemophilias – from royal genes to gene therapy. *N. Engl. J. Med.* 344(23), 1773–1779 (2001).

4 Aledort LM. Orthopedic outcome studies and cost issues. *Semin. Thromb. Hemost.* 29(1), 55–60 (2003).

5 Ljung RC. Prophylactic treatment in Sweden – overtreatment or optimal model? *Haemophilia* 4(4), 409–412 (1998).

6 Lofqvist T, Nilsson IM, Berntorp E, Pettersson H. Haemophilia prophylaxis in young patients – a long-term follow-up. *J. Int. Med.* 241(5), 395–400 (1997).

7 Nilsson IM. Experience with prophylaxis in Sweden. *Semin. Hematol.* 30(3 Suppl. 2), 16–19 (1993).

8 Mannucci PM, Giangrande PL. Choice of replacement therapy for hemophilia: recombinant products only? *Hematol. J.* 1(2), 72–76 (2000).

9 Jee YM, Go U, Cheon D *et al.* Detection of hepatitis A virus from clotting factors implicated as a source of HAV infection among haemophilia patients in Korea. *Epidemiol. Infect.* 134(1), 87–93 (2006).

10 Knight R, Stanley S, Wong M, Dolan G. Hemophilia therapy and blood-borne pathogen risk. *Semin. Thromb. Hemost.* 32(Suppl. 2), 3–9 (2006).

11 Morfini M, Azzi A, Mannucci PM. B19 parvovirus withstands "super heating" in antihemophilic concentrates. *Thromb. Haemost.* 76(5), 821 (1996).

12 Bray GL. Current status of clinical studies of recombinant factor VIII (recombinate) in patients with hemophilia A. Recombinate Study Group. *Transfus. Med. Rev.* 6(4), 252–255 (1992).

13 Rothschild C, Scharrer I, Brackmann HH *et al.* European data of a clinical trial with a sucrose formulated recombinant factor VIII in previously treated haemophilia A patients. *Haemophilia* 8(Suppl. 2), 10–14 (2002).

14 Frampton JE, Wagstaff AJ. Sucrose-formulated octocog α: a review of its use in patients with haemophilia A. *Drugs* 68(6), 839–853 (2008).

15 Tarantino MD, Collins PW, Hay CR *et al.* Clinical evaluation of an advanced category antihaemophilic factor prepared using a plasma/albumin-free method: pharmacokinetics, efficacy, and safety in previously treated patients with haemophilia A. *Haemophilia* 10(5), 428–437 (2004).

16 Toole JJ, Pittman D, Murtha P *et al.* Exploration of structure-function relationships in human factor VIII by site-directed mutagenesis. *Cold Spring Harb. Symp. Quant. Biol.* 51(Pt 1), 543–549 (1986).

■ **Experimental proof of the structure–function relationships for factor VIII and demonstrates that the B-domain is unneeded for coagulation activity.**

17 Brinkhous K, Sandberg H, Widlund L *et al.* Preclinical pharmacology of albumin-free B-domain deleted recombinant factor VIII. *Semin. Thromb. Hemost.* 28(3), 269–272 (2002).

18 Kelley BD, Tannatt M, Magnusson R, Hagelberg S, Booth J. Development and validation of an affinity chromatography step using a peptide ligand for cGMP production of factor VIII. *Biotechnol. Bioeng.* 87(3), 400–412 (2004).

■ **Proof of the feasibility of the purification process of B-domain-deleted FVIII without the requirement for mouse antibodies.**

19 Kelley BD, Booth J, Tannatt M *et al.* Isolation of a peptide ligand for affinity purification of factor VIII using phage display. *J. Chromatogr. A* 1038(1–2), 121–130 (2004).

20 Kessler CM, Gill JC, White GC *et al.* B-domain deleted recombinant factor VIII preparations are bioequivalent to a monoclonal antibody purified plasma-derived factor VIII concentrate: a randomized, three-way crossover study. *Haemophilia* 11(2), 84–91 (2005).

■■ **This was the pioneer trial demonstrating bioequivalence of moroctocog alfa and wild-type plasma-derived FVIII.**

21 Courter SG, Bedrosian CL. Clinical evaluation of B-domain deleted recombinant factor VIII in previously untreated patients. *Semin. Hematol.* 38(2 Suppl. 4), 52–59 (2001).

22 Courter SG, Bedrosian CL. Clinical evaluation of B-domain deleted recombinant factor VIII in previously treated patients. *Semin. Hematol.* 38(2 Suppl. 4), 44–51 (2001).

23 Gruppo RA, Brown D, Wilkes MM, Navickis RJ. Comparative effectiveness of full-length and B-domain deleted factor VIII for prophylaxis – a meta-analysis. *Haemophilia* 9(3), 251–260 (2003).

24 Lee ML, Morfini M. Controversial use of meta-analysis for factor VIII pharmacokinetic data. *Haemophilia* 9(6), 746–747 (2003).

25 Brown SA, Yee TT, Griffioen A, Lee CA. Comparative study of full-length and B-domain deleted factor VIII concentrates. *Haemophilia* 9(6), 745–750 (2003).

26 van der Bom JG, Fischer K, van den Berg HM. Meta-analysis on the effectiveness of B-domain deleted factor VIII for prophylaxis. *Haemophilia* 9(6), 744–750 (2003).

27 Mikaelsson M. Comparative effectiveness of full-length and B-domain deleted factor VIII for prophylaxis – another rebuttal. *Haemophilia* 10(3), 305–307 (2004).

28 Di Paola J, Smith MP, Klamroth R *et al.* ReFacto and advate: a single-dose, randomized, two-period crossover pharmacokinetics study in subjects with haemophilia A. *Haemophilia.* 13(2), 124–130 (2007).

■■ **Bioequivalence study of moroctocog alfa (ReFacto®) and octocog alfa.**

29 Ingerslev J, Jankowski MA, Weston SB, Charles LA. Collaborative field study on the utility of a BDD factor VIII concentrate standard in the estimation of BDDr Factor VIII:C activity in hemophilic plasma using one-stage clotting assays. *J. Thromb. Haemost.* 2(4), 623–628 (2004).

30 Morfini M, Cinotti S, Bellatreccia A, Paladino E, Gringeri A, Mannucci PM. A multicenter pharmacokinetic study of the B-domain deleted recombinant factor VIII concentrate using different assays and standards. *J. Thromb. Haemost.* 1(11), 2283–2289 (2003).

31 Sukhu K, Harrison P, Keeling D. Factor VIII assays in haemophilia a patients treated with ReFacto. *Br. J. Haematol.* 121(2), 379–380 (2003).

32 Santoro C, Iorio A, Ferrante F *et al.* Performance of recalibrated ReFacto laboratory standard in the measurement of FVIII plasma concentration via the chromogenic and one-stage assays after infusion of recalibrated ReFacto (B-domain deleted recombinant factor VIII). *Haemophilia* 15(3), 779–787 (2009).

■ **The most recent assessment of ReFacto laboratory standard, not anymore needed in clinical practice after ReFacto reformulation.**

33 Giel S, Blaukopf D, Daly S. A single-dose, randomized, double-blind, 2-period crossover study in patients with hemophilia A to evaluate the pharmacokinetics of B-domain deleted recombinant human Factor VIII (BDDrFVIII) manufactured by the current process (ReFacto) and by an albumin-free process (ReFacto AF). Moroctocog alfa, Pfizer internal report file, final report (2003).

■ **Randomized cross-over study demonstrating equivalence of ReFacto AF/Xyntha with the previous ReFacto formulation. This is the study that allows to carry over all the evidence about ReFacto to ReFacto AF/Xyntha.**

34 Recht M, Nemes L, Matysiak M *et al.* Clinical evaluation of moroctocog alfa (AF-CC), a new generation of B-domain deleted recombinant factor VIII (BDDrFVIII) for treatment of haemophilia A: demonstration of safety, efficacy, and pharmacokinetic equivalence to full-length recombinant factor VIII. *Haemophilia* 15(4), 869–880 (2009).

■ **Key paper in assessing ReFacto AF/Xyntha clinical profile.**

35 Windyga J, Rusen L, Gruppo R *et al.* BDDrFVIII (Moroctocog alfa [AF-CC]) for surgical haemostasis in patients with haemophilia A: results of a pivotal study. *Haemophilia* 16(5), 731–739 (2010).

36 Lusher JM, Lee CA, Kessler CM, Bedrosian CL. The safety and efficacy of B-domain deleted recombinant factor VIII concentrate in patients with severe haemophilia A. *Haemophilia* 9(1), 38–49 (2003).

37 Stieltjes N, Altisent C, Auerswald G *et al.* Continuous infusion of B-domain deleted recombinant factor VIII (ReFacto) in patients with haemophilia A undergoing surgery: clinical experience. *Haemophilia* 10(5), 452–458 (2004).

38 Smith MP, Giangrande P, Pollman H, Littlewood R, Kollmer C, Feingold J. A postmarketing surveillance study of the safety and efficacy of ReFacto (St Louis-derived active substance) in patients with haemophilia A. *Haemophilia* 11(5), 444–451 (2005).

39 Pollmann H, Externest D, Ganser A *et al.* Efficacy, safety and tolerability of recombinant factor VIII (REFACTO) in patients with haemophilia A: interim data from a postmarketing surveillance study in Germany and Austria. *Haemophilia* 13(2), 131–143 (2007).

40 Gringeri A, Tagliaferri A, Tagariello G, Morfini M, Santagostino E, Mannucci P. Efficacy and inhibitor development in previously treated patients with haemophilia A switched to a B-domain-deleted recombinant factor VIII. *Br. J. Haematol.* 126(3), 398–404 (2004).

41 Blanchette VS. Prophylaxis in the haemophilia population. *Haemophilia* 16(Suppl. 5), 181–188 (2010).

42 Berntorp E, de MP, Ljung RC. The role of prophylaxis in bleeding disorders. *Haemophilia.* 16(Suppl. 5), 189–193 (2010).

43 Iorio A , Marcucci M. Clinical trials and haemophilia: does the Bayesian approach make the ideal and desirable good friends? *Haemophilia* 15(4), 900–903 (2009).

■ **A lay reader commentary on the application of Bayesian techniques to hemophilia.**

44 Lee ML, Roth DA. A Bayesian approach to the assessment of inhibitor risk in studies of factor VIII concentrates. *Haemophilia* 11(1), 5–12 (2005).

■ **Detailed background on the currently used method to assess inhibitor rates in PTPs.**

45 Astermark J, Voorberg J, Lenk H *et al.* Impact of inhibitor epitope profile on the neutralizing effect against plasma-derived and recombinant factor VIII concentrates *in vitro*. *Haemophilia* 9(5), 567–572 (2003).

46 Khrenov AV, Ananyeva NM, Saenko EL. Role of the B-domain in proteolytic inactivation of activated coagulation factor VIII by activated protein C and activated factor X. *Blood Coagul. Fibrinolysis* 17(5), 379–388 (2006).

47 Rea C, Dunkerley A, Sorensen B, Rangarajan S. Pharmacokinetics, coagulation factor consumption and clinical efficacy in patients being switched from full-length FVIII treatment to B-domain-deleted r-FVIII and back to full-length FVIII. *Haemophilia* 15(6), 1237–1242 (2009).

■ **Observational study in UK assessing the safety of switching between octocog alfa and moroctocog alfa.**

■ Website

101 ClinicalTrials.gov http://clinicaltrials.gov/