Haemophilia (2009), **15**, 869–880 DOI: 10.1111/j.1365-2516.2009.02027.x

ORIGINAL ARTICLE *Clinical haemophilia*

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

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Summary. BDDrFVIII is a B-domain deleted recombinant factor VIII (rFVIII) product for haemophilia A. Manufacture uniquely includes purification chromatography by synthetic-affinity ligand rather than murine-based monoclonal antibody, as well as an albumin-free cell culture process. BDDrFVIII was studied in 204 patients, including 62 subjects <16 years old, in two studies. A double-blind, randomized, pharmacokinetic (PK) cross-over study, utilizing a central laboratory assay (one-stage (OS)) for both drug potency assignment and plasma FVIII-activity measurements, demonstrated that BDDrFVIII was PK-equivalent to a full-length rFVIII. Favourable efficacy and safety were observed: during defined routine prophylaxis in a patient population significant for preexisting target joints, nearly half (45.7%) of patients had no bleeding, and a low-annualized bleed rate (ABR) was achieved (median 1.9); 92.5% of haemorrhages (n = 187) required ≤2 infusions. Three subjects (1.5%, across both studies) developed de novo inhibitors (low-titre, transient), and the primary safety endpoint, based on a prospective Bayesian analysis, demonstrated the absence of neoantigenicity for BDDrFVIII. The PK-equivalence, based on central testing to align test and reference articles, and the novel Bayesian analysis of inhibitor safety in these investigations reflect robust experimental designs with relevance to future studies. This extensive dataset demonstrates the safety and efficacy of BDDrFVIII for haemophilia A.

Keywords: BDDrFVIII, factor VIII, haemophilia, pharmacokinetics

Introduction

The clinical introduction of recombinant DNA-derived clotting factor concentrates has reduced the risk of transfusion-associated infections that previously complicated use of plasma-derived replacement

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Accepted after revision 2 March 2009

therapies. Since the first recombinant factor VIII (rFVIII) was licensed, continued efforts to improve safety have led to the introduction of newer generations of rFVIII, with each successive generation representing an incremental increase in theoretical pathogen safety. The initial rFVIII products, commonly referred to as first-generation products contain human albumin in the final formulation [1]. Albumin has been eliminated from the final formulation of the second-generation products, but human and/or animal protein components are used in the cell culture processes. For what is referred to as third-generation products, all exogenous human- and animal-derived proteins have been removed from these processes. To date, murine monoclonal antibodies have been used for immunoaffinity purification of all rFVIII products [1].

Moroctocog alfa (AF-CC; Albumin Free-Cell Culture) has been developed as a successor to ReFacto, a B-domain deleted rFVIII product that was initially licensed in 1998 in Europe and 2000 in the United States. Moroctocog alfa (AF-CC), herein referred to as BDDrFVIII (B-domain deleted rFVIII), is manufactured by an albumin-free cell culture process using Chinese hamster ovary (CHO) cells grown in a chemically defined serum-free cell culture medium that contains no materials derived from human or animal sources [2]. A synthetic peptide affinity ligand (TN8.2) replaces the murine monoclonal antibody used in ReFacto affinity chromatography, and an additional 35 nm viral filtration step is added during purification, expanding upon the viral-safety programme currently in use for ReFacto [3].

BDDrFVIII was manufactured by the albumin-free cell culture process for clinical use using two different potency assignment processes. One has been developed for the United States, Canada and certain other regions of the world, with its potency assignment aligned to the one-stage (OS) FVIII activity assay. This product is identified by the trade name Xyntha™. The other, identified as ReFacto AF™, has been developed for the European Union and other regions of the world in accord with the European Pharmacopoeia, with its potency assignment aligned to the chromogenic substrate (CS) FVIII activity assay. The rationale for developing different potency assignment processes for Xyntha and for ReFacto AF was based on the objective, for the former, to align the potency assignment of BDDrFVIII with the more commonly used OS clinical monitoring assay in regions of the world where regulatory requirements were permissive for this objective. This objective necessitated an extensive clinical programme encompassing several global studies with each product. An initial clinical study using BDDrFVIII with a potency assignment aligned to the CS assay demonstrated pharmacokinetic (PK) equivalence to ReFacto and provided the necessary clinical evidence that the manufacturing modifications associated with the albumin-free cell culture process, including introduction of the synthetic peptide ligand affinity purification and a viral filtration step, had no impact on the PK profile of the predecessor product, ReFacto [4]. To extend these observations to the clinical safety and efficacy of BDDrFVIII with a potency assignment aligned to either the CS or the OS assay, two subsequent global clinical studies, collectively enrolling 204 previously treated patients (PTPs) with severe or moderately severe haemophilia A, were initiated. The outcomes of these investigations, the largest clinical experience to date supporting development and registration of a rFVIII, are the focus of this report.

Patients and methods

The clinical trials were approved by each respective institutional review board or ethics committee and were conducted according to good clinical practice. All participants gave written informed consent according to the Declaration of Helsinki.

The more recently completed study is herein referred to as Study 1 and is presented first.

Study 1

Clinical protocol design, patients and procedures Study 1 was conducted using BDDrFVIII with a potency aligned to the OS assay and calibrated to the WHO Seventh International Standard for Blood Coagulation FVIII:C Concentrates. The primary objectives were to demonstrate PK-equivalence of BDDrFVIII and a full-length rFVIII concentrate (FLrFVIII, Advate™, Baxter) by the standard equivalence criteria, using the OS clotting assay, and to demonstrate safety of BDDrFVIII with respect to inhibitor development. The initial PK period included a randomized, double-blind, crossover study to assess PK-equivalence of BDDrFVIII and FLrFVIII in at least 24 patients with severe haemophilia A (FVIII activity [FVIII:C] $\leq$1 IU dL^{-1}). This was followed by an open-label, 6-month safety and efficacy (S&E) period evaluating BDDrFVIII for prevention of haemorrhages and for on-demand treatment in at least 81 PTPs with severe or moderately severe haemophilia A (FVIII:C $\leq$2 IU dL^{-1}), including the patients participating in the initial PK period. Patients participating in the initial crossover PK assessments were to complete

the S&E period and then participate in a 6-month follow-up PK assessment using BDDrFVIII. At study entry, all patients were ≥12 years old, had ≥150 prior exposure days (ED) to any FVIII product, and had negative assays for FVIII inhibitor at both local and central laboratories. Additional inclusion criteria were: normal liver and kidney function, platelet count ≥100 000 μL^{-1}, absolute CD4 count >400 μL^{-1}, and normal prothrombin time or INR ≤1.5. Patients receiving therapy for HIV or hepatitis had to be on a stable regimen. All patients had to be able to withhold FVIII infusions for ≥72 h prior to each FVIII activity and inhibitor assay. Exclusion criteria included: history of FVIII inhibitors (≥0.6 Bethesda Units [BU] mL^{-1}), additional bleeding disorder(s), use of an investigational drug/device ≤30 days prior to study entry, regular use of antifibrinolytics or medications affecting platelet function, use of immunosuppressive drugs, history of hypersensitivity to hamster protein, and elective surgery planned to occur during the study. The clinical study began in June, 2005 and the final subject completed the study in November 2006.

Treatment For the PK studies, patients received a 50 IU kg^{-1} infusion, based upon the manufacturer's labelled potency, of BDDrFVIII followed by FLrFVIII, or in the reverse order, based on a prospectively randomized treatment-sequence assignment; four lots of each product were evaluated. Both PK infusions were to occur within a 28-day interval with a ≥72-h washout following prior FVIII administration. Patients returned following 6 months of S&E treatment to receive a single infusion (50 IU kg^{-1}) of BDDrFVIII for final follow-up PK assessment.

During the S&E period, BDDrFVIII was routinely administered to prevent bleeding episodes using the same regimen (30 ± 5 IU kg^{-1}, three times a week) for all patients for ≥50 EDs. Dose escalation to 45 ± 5 IU kg^{-1}, three times a week was prespecified if two spontaneous haemorrhages occurred in a major joint, or if ≥3 spontaneous haemorrhages occurred in any location over a 28-day period. All haemorrhages were treated with BDDrFVIII at the discretion of the investigator (or by the patient with investigator guidance) based on protocol guidelines following standard treatment practises. Surgery was not permitted during this study.

Pharmacokinetic analyses PK specimens were collected to determine FVIII:C in patient plasma prior to each infusion and then at 0.25, 0.5, 1, 3, 6, 9, 24, 28, 32 and 48 h postinfusion. All FVIII:C determinations were performed by a central laboratory (Covance Laboratories, Chantilly, VA, USA), using a validated OS clotting assay calibrated with the Dade Plasma Standard. To account for potential differences in methods of potency determination by manufacturers of BDDrFVIII and FLrFVIII, the potency of the two PK study drugs was determined head-to-head in the same OS clotting assay by the central laboratory. The use of the same assay in the central laboratory also assured alignment between the method for determination of FVIII:C in patient plasma and the method of potency assignment, and was consistent with regulatory guidance for the development of this study. Levels of FVIII:C in patient plasma were adjusted for preinfusion activity level according to standard guidelines for analysis of PK studies of coagulation factors [5] and results were normalized to a dose of 50 IU kg^{-1}. The primary analysis of PK equivalence of BDDrFVIII versus FLrFVIII was based on the central laboratory potency assessment in accordance with regulatory guidance that was provided during protocol development. Secondary analyses of BDDrFVIII PK parameters at baseline and following 6 months of treatment were also performed based on the manufacturer's labelled potency. Descriptive statistics of the estimated PK parameters were calculated. Comparisons of the primary PK parameter estimates (AUC_t, AUC_∞, *K*-value) between BDDrFVIII and the comparator FLrFVIII were performed using an analysis-of-variance for a two-period crossover design, utilizing the statistical method previously described [6]. Additionally, 90% confidence intervals (CI) for the test-to-reference ratios of the primary PK parameter estimates were constructed on the log-scale using the two one-sided tests procedure. BDDrFVIII and FLrFVIII were determined to be PK-equivalent if the 90% CIs of the ratio of geometric least squares means of the primary PK parameters fell within the equivalence window of 80–125% [5,7]. Similarly, BDDrFVIII PK properties were considered unchanged if the primary PK parameter estimates at month 6 and baseline met these criteria. Calculation of PK parameters for the respective study drugs was performed as previously described [5,6,8].

Efficacy assessments Efficacy of BDDrFVIII during the S&E portion was assessed by the percent of patients experiencing haemorrhages and by the annualized bleed rate (ABR), during the protocol-specified routine prophylaxis. Efficacy of each BDDrFVIII infusion for on-demand treatment was assessed by the number of doses required to treat the haemorrhage, and by the patient/guardian using a four-point scale (definitions, Fig. 4) [9,10].

The frequency of less-than-expected therapeutic effect (LETE) during prophylaxis (spontaneous haemorrhage ≤48 h of scheduled dose) and in the on-demand setting ('no response' rating, per the four-point scale, after each of two successive infusions ≤24 h), both in the absence of confounding factors, was calculated.

Safety assessments Inhibitor testing was conducted at the central laboratory (Nijmegen assay) and defined by a titre of ≥0.6 BU mL^{-1}. Samples were collected at screening, and months 0, 1, 3 and 6. Safety was also assessed by adverse events (AE), and clinical and laboratory evaluations. Testing for presence of antiBDDrFVIII, antiCHO and anti-TN8.2 antibodies was performed at a central laboratory (Covance Laboratories, Chantilly, VA, USA) using validated ELISA assays.

Study 2

Study 2 was conducted using BDDrFVIII with a potency aligned to the CS assay, and calibrated to the WHO Sixth International Standard for Blood Coagulation FVIII:C Concentrates. Objectives were similar to those of study 1, and also included characterization of safety and efficacy during surgery. This study was open-label, and subjects were to accrue ≥50 BDDrFVIII EDs. Treatment included BDDrFVIII prophylaxis infusions at least twice a week (except patients continuing from a preceding crossover-study assessing the PK-equivalence of BDDrFVIII and ReFacto; these patients could be treated solely on an on-demand basis at the investigator's choice). All regimens, including surgical treatment if necessary, were determined by investigators. Eligibility criteria matched study 1 with the following exceptions for inclusion: prior FVIII exposure ≥250 EDs, and ≥6 years old. Efficacy and safety assessments were similar to those described for study 1. Study 2 began in April, 2002 and the final patient completed the study in August 2004.

Statistical analysis

Safety and efficacy data for studies 1 and 2 were summarized using descriptive statistics. Study 1 included a prespecified primary safety endpoint to assure the absence of neoantigenicity associated with the manufacturing changes used to develop BDDrFVIII. A Bayesian statistical model was developed to assess the primary safety endpoint of inhibitor development. A detailed explanation of the Bayesian model and its utility for assessment of inhibitor risk in studies of FVIII concentrates has been published [11]. This model was adapted for use in study 1. The maximum population inhibitor rate based on an intent-to-treat (ITT) analysis that would be considered clinically acceptable, and which would define an upper threshold limit for defining the success or failure of the study 1 primary safety endpoint, was determined based on existing data at the time for licensed FLrFVIII products. A Beta distribution was used to model the probability of inhibitor development. Published data on the pivotal registration trials for the four licensed FLrFVIII products reported the development of six inhibitors in a total of 329 patients by ITT (Kogenate, 2/86; Recombinate, 2/69; Kogenate FS, 1/71; Advate, 1/103) [11]. A non-informative (uniform) prior distribution of Beta (1,1) was, therefore, updated with these data using the formula for a posterior Beta $(x + 1, n - x + 1)$ distribution, with x = number of inhibitors and n = number of subjects. This generated the standard inhibitor rate probability distribution for FLrFVIII products of Beta (7,324). The mean rate of this distribution was 2.1% and the 99th percentile of the distribution was chosen to define the upper threshold limit for clinical acceptance. This threshold limit was observed at an inhibitor rate of 4.4%. To analyze the inhibitor rate in study 1, a Beta distribution was also used to model the prior probability of inhibitor development for BDDrFVIII based on available data for related B-domain deleted rFVIII products. Published data on the pivotal registration trials for ReFacto reported the development of one inhibitor in 113 patients [12]. In study 2, one de novo inhibitor case and two cases of patients with recurrent low-titre inhibitors were observed in 110 patients. Thus, the pooled data for B-domain deleted rFVIII products by ITT included four inhibitors in a total of 223 patients. Using these data, a uniform prior distribution of Beta (1,1) was updated to a BDDrFVIII inhibitor rate probability distribution of Beta (5,220) with a mean of 2.2%. Since study 1 aimed to enrol over 80 evaluable patients, this BDDrFVIII prior probability distribution was discounted 50% to a probability distribution of Beta (2.5,110), to align the extent of the prior data with the anticipated size of study 1. The mean of the discounted BDDrFVIII prior probability distribution was also 2.2%, similar to the mean of the standard (FLrFVIII) inhibitor rate probability distribution used to set the upper threshold of 4.4%. In practical terms, a maximum of two inhibitors in 81 subjects could be observed by ITT to satisfy the primary safety endpoint, and to demonstrate the population

Table 1. Demographic and baseline characteristics for subjects treated with BDDrFVIII.

	Study 1 (*N* = 94)	Study 2 (*N* = 110)
Age (years), Median (range)	24 (12–60)	19.0 (7–70)
Age category, *n* (%)		
<16 years	17 (18.1%)	45 (40.9%)
≥16 years	77 (81.9%)	65 (59.1%)
Race, *n* (%)		
White	89 (94.7%)	95 (86.4%)
Other	5 (5.3%)	15 (13.6%)
HIV status positive	9 (9.6%)	15 (13.6%)
Hepatitis C status positive	66 (70.2%)	53 (48.1%)
Target joints: Yes	74 (78.7%)	NR

HIV, human immunodeficiency virus; NR, not reported.

inhibitor rate was below the 4.4% threshold with ≥95% probability.

Results

Subjects

A total of 94 and 110 subjects were treated with BDDrFVIII in studies 1 and 2, respectively. Demographics for these two study populations were similar (Table 1). Medical history information captured in study 1 revealed that 79% of subjects had preexisting target joints. Study 2 included a wider age range of subjects, due to the eligibility criteria allowing younger patients to participate.

The percentage of subjects completing each study was high (96% and 94% for studies 1 and 2, respectively), with median participation durations of 34.4 weeks (range, 21.3–43) and 22.5 weeks (range, 4.1–78), respectively. During the course of study 1, 6775 infusions of BDDrFVIII were administered for PK assessments, prophylaxis and on-demand treatment; during study 2, 7035 infusions were administered for these same purposes and for surgical prophylaxis as well (Table 2).

Pharmacokinetics, pharmacokinetic equivalence and stability

The direct, comparative PK evaluation between FLrFVIII and BDDrFVIII in study 1 was based upon the central laboratory potency assessment for the respective products and showed nearly identical mean FVIII activity-versus-time profiles (Fig. 1). Analysis of PK parameters for BDDrFVIII and FLrFVIII demonstrated, in 30 subjects, that the two products were PK-equivalent: 90% CIs about the ratios of BDDrFVIII to FLrFVIII geometric least squares means of the key PK parameters (AUC_t, AUC_∞, *K*-value) were all within the PK-equivalence window of 80–125% (Table 3). Similar *in vivo* recovery estimates were also found.

Baseline BDDrFVIII PK parameters were also estimated based on the manufacturer's labelled potency (Table 4). These PK parameters remained unchanged with repeated BDDrFVIII use over time in 25 subjects: 90% CIs about the ratios of BDDrFVIII month 6-to-baseline geometric least squares means of key parameters were all within the 80–125% equivalence window (Table 4).

Table 2. (a) Summary of BDDrFVIII exposure, (b) Bleeding rates during BDDrFVIII routine prophylaxis.

	Total units (IU)	Total no. infusions	Total no. EDs	No. (%) of subjects with ≥50 EDs
(a)				
Study 1 (*N* = 94)	15302078*	6775	6741	89 (95)
Study 2 (*N* = 110)	16233964†	7035	6860	99 (90)
Studies 1 and 2		13810	13601	188 (92)

	Study 1 (*N* = 94)	Study 2 (*N* = 104)
(b)		
Prophylaxis regimen	Defined	Undefined
Infusion frequency	3 per week	2 to >3 per week
Median dose per infusion (IU kg^{-1})	30.2	31.4
Mean (±SD) duration of routine prophylaxis (weeks)	24.6 ± 3.7	22.4 ± 7.4
Subjects with no haemorrhages	43 (45.7%)	25 (24.0%)
Subjects with no spontaneous haemorrhages	57 (60.6%)	51 (49.0%)
Annualized bleeding rate‡		
Median (range)	1.9 (0 – 42.1)	5.2 (0 – 44.7)
Mean ± SD	3.9 ± 6.5	7.7 ± 8.6

ED, exposure day (any calendar day on which BDDrFVIII was received); No., number.
*BDDrFVIII potency assignment by OS assay.
†BDDrFVIII potency assignment by CS assay.
‡All haemorrhages including spontaneous and injury-related haemorrhages.

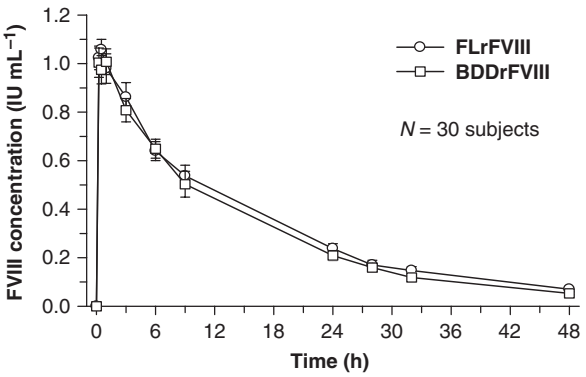

Fig. 1. Mean (±SE) factor VIII activity-versus-time profiles following a 50 IU kg^{-1} infusion of FLrFVIII or BDDrFVIII based on the central laboratory potency assessment.

Haemostatic efficacy

In study 1, 94 subjects initiated routine prophylaxis according to the protocol-defined regimen (30 IU kg^{-1}, three times per week). A total of 6347 infusions (14 194 228 IU total) of BDDrFVIII, were administered for routine prophylaxis. During the protocol-defined regimen, 43 (45.7%) of subjects experienced no bleeding episodes. In addition, 14 subjects had no spontaneous haemorrhages; thus, more than half of subjects (60.6%) had no spontaneous haemorrhages during their course of BDDrFVIII routine prophylaxis. Fifty-one subjects reported a total of 180 haemorrhages during routine prophylaxis: 110 episodes occurred ≤48 h and 70 episodes occurred >48 h after the last prophylaxis infusion. Most haemorrhages occurring within the initial 48 h were caused by injury (58%), whereas the majority of haemorrhages occurring after the initial 48 h (60%) were spontaneous. This observation is consistent with the expected decline of FVIII activity over time. Of the total 46 spontaneous bleeds occurring within 48 h after the last BDDrFVIII prophylaxis infusion, 25 were judged as meeting the predefined criteria for LETE (incidence of 0.4%; 25 events per 6347 routine prophylactic infusions). Dose escalations were minimal, with only six patients (6.4%) requiring a regimen change. Overall, routine prophylaxis, as defined in study 1, afforded a low mean ABR of 3.9 (median = 1.9, Table 2).

Table 3. Parameters for PK-equivalence testing of BDDrFVIII and FLrFVIII based upon central laboratory product potency assessments.

	AUC_t (IU h mL^{-1})	AUC_∞ (IU h mL^{-1})	*K*-value ([IU dL^{-1}]/[IU kg^{-1}])	*In vivo* recovery (%)
BDDrFVIII*	13.8 ± 5.7	14.7 ± 6.1	2.35 ± 0.47	112 ± 22
FLrFVIII*	15.0 ± 5.4	16.5 ± 6.3	2.39 ± 0.65	114 ± 30
Geometric LS mean ratio (%)	89.8%	88.0%	100%	ND
90% log-transformed CI	83.3–96.9%	81.6–94.8%	92.5–108%	ND

*N = 30 subjects; mean ± standard deviation.

AUC_t, area under the plasma concentration–time curve from time zero to the last measurable activity; AUC_∞, area under the plasma concentration–time curve from time zero to infinity; CI, confidence interval; *K*-value, incremental recovery; LS, least squares; ND, not done, PK-equivalence testing restricted to AUCs and *K*-value; PK, pharmacokinetic.

Table 4. PK parameter estimates for BDDrFVIII at baseline, and PK stability over time testing, based upon labelled potency.

	Baseline*	Month 6-to-baseline testing*	
	Mean ± sd	Geometric LS mean ratio (%)	90% log-transformed CI
AUC_t (IU h mL^{-1})	12.7 ± 5.2	100	89.2–112.2%
AUC_∞ (IU h mL^{-1})	13.5 ± 5.6	104	93.9–115.4%
K-value (IU dL^{-1} per IU kg^{-1})	2.15 ± 0.44	107	96.4–119.6%
C_{max} (IU mL^{-1})	1.08 ± 0.22	ND	
$t_{1/2}$ (h)	11.2 ± 5.0	ND	
CL (mL h^{-1} kg^{-1})	4.51 ± 2.23	ND	
In vivo recovery (%)	103 ± 21	ND	

*Baseline, *N* = 30 subjects; Month 6-to-baseline testing, *N* = 25 subjects.

AUC_t, area under the plasma concentration–time curve from time zero to the last measurable activity; AUC_∞, area under the plasma concentration–time curve from time zero to infinity; CI, confidence interval; CL, clearance; C_{max}, peak concentration; *K*-value, incremental recovery; LS, least squares; ND, not done, PK-equivalence testing restricted to AUCs and *K*-value; PK, pharmacokinetic; SD, standard deviation; $t_{1/2}$, elimination half-life.

On-demand BDDrFVIII treatment was administered for haemorrhages occurring during and prior to initiation of routine prophylaxis. In total, 187 haemorrhages, occurring in 53 subjects, were resolved with 282 infusions (658 004 IU total; median dose: 30.6 IU kg^{-1}). The most common haemorrhage locations were joint (61%) or soft tissue/muscle (23%) sites. Over 90% of haemorrhages (92.5%) were resolved with ≤2 infusions of BDDrFVIII, with most requiring only a single infusion (74.3%; Fig. 2). These outcomes were not limited to a particular haemorrhage site, but instead were common to all haemorrhage locations (Fig. 3). Response to the first BDDrFVIII infusion used to initiate treatment was rated *Excellent* or *Good* for 70.6% of haemorrhages. These ratings were not restricted to infusions with higher doses, as even the majority of initial infusions within the lowest dose categories (<20 IU kg^{-1} and 20–30 IU kg^{-1}) yielded responses of *Excellent*/*Good* (Fig. 4). The incidence of LETE in the on-demand setting was low (0.5%; 1 event per 187 haemorrhages).

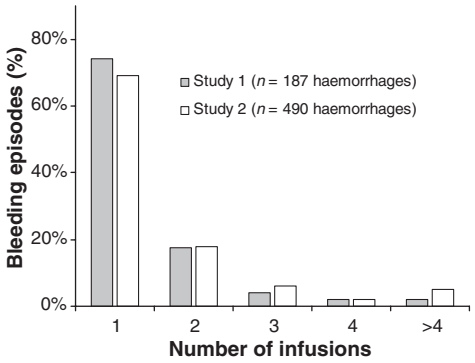

Fig. 2. Number of BDDrFVIII infusions required for resolution of haemorrhages in study 1 and study 2.

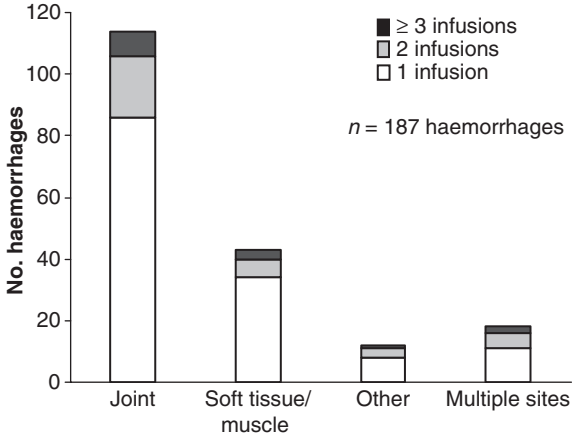

Fig. 3. The number of haemorrhages resolved by 1, 2, or ≥3 infusions by haemorrhage site (Study 1).

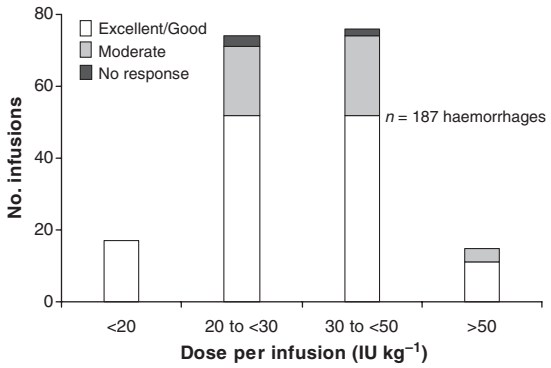

Fig. 4. Response rating to first BDDrFVIII infusion used to initiate treatment of a haemorrhage as a function of dose (Study 1). Ratings defined as follows: Excellent – abrupt pain relief and/or improvement in signs of bleeding within approximately 8 h after a single infusion; Good – definite pain relief and/or improvement in signs of bleeding within approximately 8 h after an infusion, but possibly requiring more than one infusion for complete resolution; Moderate – probable or slight beneficial effect within approximately 8 h after the first infusion; usually requiring more than one infusion; or No Response – no improvement, or condition worsens. (Response rating for five haemorrhages not reported).

In study 2, 104 subjects used BDDrFVIII for routine prophylaxis according to investigator-prescribed regimens, and six subjects used BDDrFVIII on an unscheduled, as-needed basis. Overall, results were similar to study 1. BDDrFVIII was effective in prevention and treatment of haemorrhages: 24% of patients had no haemorrhages and 49% of patients had no spontaneous haemorrhages during routine prophylaxis; the mean ABR during prophylaxis was 7.7 (median = 5.2, Table 2); most haemorrhages (425 of 490, 86.7%) resolved with ≤2 infusions (Fig. 2; median dose, 37.6 IU kg^{-1}); the majority of first infusions administered for haemorrhage treatment were rated *Excellent*/*Good* [413 of 482, 86% (eight not reported)]; and the incidence of LETE was low in both the routine prophylaxis and on-demand settings (0.09% and 0.2%, respectively). Use of BDDrFVIII for surgical support was allowed; nine patients received a cumulative total 328 155 IU for this purpose. Procedures included: closed reduction and internal fixation of arm fracture, ankle arthroscopy, two dental extractions, incision and drainage of an abscess, an unspecified arthroscopic procedure, two ankle arthrodesis, and radial head resection. In all cases, haemostasis was managed with BDDrFVIII, blood loss was minimal (≤50 mL), and no transfusions were required.

Safety

In total, 204 patients acquired 13 601 BDDrFVIII EDs during studies 1 and 2, with >90% of subjects achieving ≥50 BDDrFVIII EDs (Table 2).

The primary safety endpoint of study 1 to demonstrate an inhibitor rate below the 4.4% threshold with ≥95% probability was achieved using a Bayesian statistical analysis (Fig. 5). The observed incidence of FVIII inhibitor development was 2.1% in the ITT population (2 of 94 subjects), and 2.2% (2 of 89 subjects) in the per-protocol population restricted to subjects with ≥50 EDs. Analyses of each of these patient populations passed the prespecified primary safety endpoint: the maximum rate for inhibitor development was less than 4.07% and less than 4.17% with ≥95% probability for the ITT and per-protocol populations, respectively. Specifically, transient low-titre FVIII inhibitors were detected in two subjects (single time-point detections: 0.98 BU mL^{-1} after 38 BDDrFVIII EDs in one subject; 1.2 BU mL^{-1} after 81 EDs in the other subject). Neither patient had associated clinical symptoms or evidence for reduced efficacy of BDDrFVIII treatment associated with the laboratory-based diagnosis. In study 2, low-titre FVIII inhibitors (<5 BU mL^{-1}) were detected in three subjects; only one case was consistent with development of a de novo inhibitor, while historical inhibitor testing for the other two subjects indicated these to be recurrent cases. One patient with a recurrent inhibitor was enrolled in violation of protocol entry criteria, while the other patient was eligible despite multiple measurements of low-titre inhibitors, just below the 0.6 BU mL^{-1} cut-off. Follow-up reporting for the single de novo case indicated the low-titre inhibitor had resolved following a short 3-month course of immune tolerance induction using BDDrFVIII. ELISA testing for anti-BDDrFVIII immune response yielded negative results for the two inhibitor patients in study 1, but positive results for the three inhibitor cases in study 2.

AE reporting and laboratory testing revealed no other evidence for clinically significant immune responses to BDDrFVIII. Per AE reporting, no patients had clinical allergic reactions to BDDrFVIII in either study. AEs judged related to BDDrFVIII treatment by the investigator in study 1 included asthenia, arthralgia, and haemorrhage; all these events were reported in the same patient, were of mild severity, and resolved. Related events in study 2 included those reported in study 1 [asthenia (one subject) and arthralgia (four subjects)] and also included joint disorder (two subjects), onset of cyst, headache, injection site reaction, nausea, ecchymosis, splenomegaly, myalgia, confusion, and taste perversion (one subject each). Of these, cases of arthralgia (two subjects), joint disorder (one subject), and cyst were judged to be severe. Analysis of BDDrFVIII-related AEs showed no clinically significant difference in frequency of events across age groups (6–11, 12–16, and 17–65 years). In summary, 3 (3%) subjects in study 1 and 11 (10%) subjects in study 2 had AEs judged related to BDDrFVIII treatment, including FVIII inhibitor development. In laboratory testing for immunogenic response, ELISA results were negative for immune response to CHO proteins or TN8.2 during both studies. AntiBDDrFVIII ELISA positivity was found in two subjects (across both studies) who did not have FVIII inhibitor and who did not have any clinical symptoms indicative of immunogenic response, including reduced efficacy of BDDrFVIII treatment. The only other anti-BDDrFVIII immune responses by ELISA were found for the three inhibitor cases in study 2, noted above.

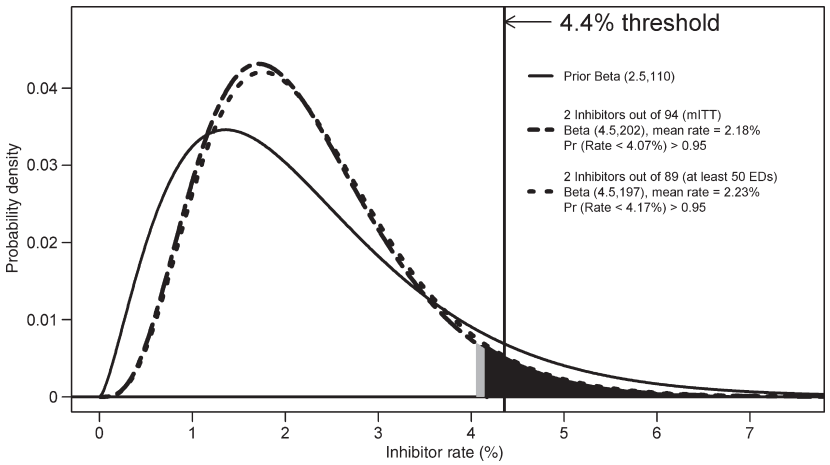

Fig. 5. Prior and posterior Beta probability distributions for inhibitor rate. Distributions are shown for the total patient population in study 1 (N = 94 subjects; dashed line) and for the subset of patients in study 1 who accrued ≥ 50 exposure days (N = 89 subjects; dotted line).

Discussion

BDDrFVIII has been shown to be PK-equivalent to FLrFVIII using the OS assay with PK parameters that are stable after 6 months of treatment. The experimental design for the demonstration of PK-equivalence is a robust one. Manufacturer's processes may vary with respect to calibration of standards and assay methodology. The use of the same FVIII:C assay in the central laboratory to determine the potency of each FVIII drug assures that the demonstration of PK-equivalence is independent of manufacturer's process for assignment of potency. Furthermore, use of the identical assay for determination of administered drug potency and of resultant plasma FVIII:C assures that the conclusions are independent of assay method (OS or CS); any correction factor for use of a different assay would apply equally to the dose of FVIII administered, which is derived from the potency, and to the direct determinations of plasma FVIII:C. Although not specifically part of published guidance documents regarding studies of PK-equivalence [5,13], these study design features should be strongly considered in future comparative studies in order to eliminate any bias based upon manufacturer's method of potency assignment and to assure independence of PK-equivalence findings from method of FVIII assay.

These investigations also demonstrate that routine scheduled infusions of BDDrFVIII are effective in preventing haemorrhages in patients who commonly (79% of subjects) have preexisting target joint(s). During defined routine prophylaxis with BDDrFVIII, a mean ABR of 3.9 was observed ($N = 94$). This result aligns with outcomes from other studies also assessing regular infusions of FVIII. For example, a mean ABR of 6.3 was reported during prophylaxis of patients age ≥10 years using a third-generation FLrFVIII [10], and an ABR of 4.2 was determined in a retrospective study evaluating secondary prophylaxis in adolescent and adult patients with severe haemophilia A and B [14]. The mean ABR of 7.7 observed in study 2 during routine prophylaxis with BDDrFVIII also aligns with these results. Differences in the ABR observed in studies 1 and 2 can be explained by the less structured regimen in study 2, the lack of prespecified criteria for dose escalation in study 2, and the possibility of investigator bias in assigning regimens during study 2. The favourable impact of a more structured regimen was also seen with FLrFVIII [10]; mean ABRs of 4.4 and 9.9 were observed in patients adherent versus non-adherent to the prescribed regimen, respectively. The inclusion of younger patients in study 2 could also be a contributing factor for the observed difference in ABRs for studies 1 and 2, as younger children are likely to be more susceptible to injury-related bleeding associated with play. In study 2, 40.9% of patients were <16 years old, including patients as young as 7 years, whereas 18.1% of study 1 patients belonged to this age category and none were below 12 years of age (Table 1).

The efficacy of BDDrFVIII for on-demand treatment was also consistently demonstrated in each of these two global studies. The similarly favourable outcomes confirm that BDDrFVIII efficacy is independent of drug potency assignment method (OS and CS assays used in studies 1 and 2, respectively). In study 1, 92.5% of haemorrhages resolved with ≤2 infusions of BDDrFVIII, with most (74.3%) requiring only a single infusion. Results from study 2 and aggregate results across the two studies are consistent with these findings; collectively, nearly all (88%) of the 677 haemorrhages which occurred in studies 1 and 2 were controlled with ≤2 infusions.

Efficacy, as assessed by LETE, was also evaluated in these studies. LETE is recognized for all FVIII concentrates. The occurrence of clinical outcomes consistent with LETE (as defined in these studies) is demonstrated in published clinical data of available rFVIII products [15,16]. While the specific frequency of LETE is not reported in the published literature, data is consistent with the occurrence of spontaneous bleeding within 48 h of prophylactic dosing. For example, a mean spontaneous ABR of 3.3 in the setting of a ≥3 per week FLrFVIII schedule has been reported [10]. Other reports indicate that approximately 1–10% of on-demand infusions are not effective based on the occurrence of 'no response/worse/not successful' ratings [15,16]. Even with prospective collection of LETE in studies 1 and 2, occurrence was very infrequent in both the routine prophylaxis and on-demand settings (incidences of ≤0.4% and ≤0.5%, respectively, during each study). Overall, BDDrFVIII successfully prevented and controlled haemorrhages in 204 haemophilic patients affected with severe or moderately-severe forms of the disease.

These studies also confirm the inhibitor safety of BDDrFVIII and indicate that the manufacturing modifications for the new BDDrFVIII albumin-free cell culture process were not associated with neoantigenicity of the BDDrFVIII product. In study 1, in accord with its primary prespecified safety endpoint, only two instances of inhibitor, both transient and clinically silent, were observed in 94 patients, including 89 patients with ≥50 EDs while on study. The assessment of inhibitor risk in clinical trials of

new or modified rFVIII products is challenging, due to the low frequency of inhibitor occurrence and the generally small size of haemophilia studies due to the rarity of the disease. The paradigm used in prior haemophilia studies to date has relied on assessment of the point estimate of inhibitor occurrence, with interpretation of clinical significance focused on de novo inhibitors in patients with no prior inhibitor history. As the infectious safety of FVIII products has largely been achieved with current day recombinant protein manufacturing technologies, the neoantigenicty of a new or modified recombinant product has become a primary focus of contemporaneous clinical investigations of new haemophilia therapies. Study 1 included a prospectively defined primary safety endpoint for assessment of inhibitor risk to address expectations for increased rigor associated with safety assessments of new or modified haemophilia products. Statistical modelling of inhibitor safety using traditional approaches based on CIs have challenges associated with the generally small sample size of haemophilia registration trials and the complexities of inhibitor determination. These challenges are based on interpretation of the clinical significance of the observed inhibitor, the clinical outcome for the affected patient, the patient's underlying risk for inhibitor development, and the inhibitor testing method and testing frequency during the investigation [11]. An alternative Bayesian statistical approach [11], based on probability assessments of inhibitor development and informed by a systematic assessment of prior data from relevant clinical trials, is sensitive, flexible, clinically intuitive and well-suited to evaluate a low-incidence safety endpoint in small clinical studies. Study 1 represents the first prospective use of this Bayesian approach to demonstrate inhibitor safety in the context of a FVIII clinical registration trial. The study analysis demonstrated that the maximum rate for inhibitor development following BDDrFVIII treatment was less than the prespecified primary safety endpoint for both the ITT analysis of all 94 treated patients and for the per-protocol analysis restricted to the 89 subjects with ≥50 EDs.

There was no other evidence of significant neoantigenicity for BDDrFVIII during studies 1 and 2. In AE reporting, there were no cases of allergic reaction to BDDrFVIII, and laboratory testing showed no evidence of immunogenic response to BDDrFVIII manufacturing components, including the production cell line (CHO) and the synthetic purification ligand (TN8.2). Two subjects (one in each study), who did not have inhibitors, had evidence for anti-BDDrFVIII immune responses by ELISA. The significance of antiBDDrFVIII ELISA positivity for these two subjects in the absence of FVIII inhibitor, or reduced efficacy of BDDrFVIII treatment, or other clinical symptoms indicative of immunogenic response is unknown.

Overall, BDDrFVIII was well-tolerated. Collectively, only 14 of 204 subjects (7%) had AEs deemed related (or possibly related) to BDDrFVIII treatment [three in study 1 (3%) and 11 in study 2 (10%), inclusive of subjects with FVIII inhibitor]. In general, a number of these related events were consistent with complications of haemophilia (e.g. arthralgia, ecchymosis, joint disorder), and nearly all events were of mild or moderate severity.

Conclusions

In conclusion, this new generation BDDrFVIII product has been shown to be PK-equivalent to FLrFVIII with PK parameters that are stable over time. BDDrFVIII is a pathogen-free source of FVIII, shown to be effective and safe for haemophilia A treatment. Efficacy of BDDrFVIII in the prevention and treatment of haemorrhages is consistent across studies, regardless of method for potency assignment, and aligns with published literature for other FVIII products [8,10,17]. These investigations have been conducted in a manner which is highly instructive for PK study design and which offer proof-of-concept for the use of Bayesian methodology to demonstrate inhibitor safety. These designs for both PK-equivalence testing and for inhibitor safety, should be considered in future clinical studies. In particular, these designs have relevance for studies of new coagulation proteins that may be engineered to enhance efficacy/safety of currently available therapies for haemophilia and other clotting-factor deficiencies.

Acknowledgements

This study was sponsored by Wyeth. The contributions of the study investigators and their respective study sites, listed below, are greatly appreciated: Thomas C. Abshire – Emory University, Department of Pediatrics and Medicine, Atlanta, GA; Steven Arkin – Long Island Jewish Medical Center, New Hyde Park, NY and Mount Sinai Medical Center, NY; Ross Baker – Royal Perth Hospital, Perth, Australia; Zale P.Bernstein – Hemophilia Center for Western New York, Buffalo, NY; Erik Berntorp – Department for Coagulation Disorder, Malmö, Sweden; John D. Bouhasin – SSM Cardinal Glennon Hospital, St. Louis, MO; Howard Britton – South

Texas Community Hemophilia Center, San Antonio, TX; Deborah Brown – Children's Memorial Hospital, Chicago IL; Elizabeth Chalmers – Royal Hospital for Sick Children, Glasgow, United Kingdom: Alicja Chybicka – Academic Clinical Hospital, Wroclaw, Poland; Alice J. Cohen – Newark Beth Israel Medical Center, Newark, NJ; Peter Collins – University Hospital of Wales, Cardiff, United Kingdom; Donna DiMichele – The New York Hospital-Cornell Medical Center, New York, NY; Jorge DiPaola – University of Iowa Hospital and Clinic, Iowa City, IA; M. Elaine Eyster – Penn State University, Hershey, PA; James A. French II – The Children's Medical Center, Dayton, OH; Ralph Gruppo – Children's Hospital Medical Center, Cincinnati, OH; Ian Hann – Great Ormond Street Hospital For Children, London, United Kingdom; Paul Harper – Auckland City Hospital, Auckland, New Zealand; Charles Hay -Manchester Royal Infirmary, Manchester, United Kingdom; Margareta Holmström – Karolinska University Hospital-Solna, Stockholm, Sweden; Keith Hoots – University of Texas, Houston Medical School, Houston, TX; Craig Kessler – Georgetown University Medical Center, Washington, DC; Robert Klamroth – Clinical Center in Friedrichshain, Berlin, Germany; Barbara Ann Konkle – University of Pennsylvania School of Medicine, Philadelphia, PA; Riitta Lassila – Helsinki University Central Hospital, Helsinki, Finland; Jeanne Lusher – Children's Hospital of Michigan, Detroit, MI; Alice Ma – University of North Carolina at Chapel Hill, Chapel Hill, NC; Marilyn Manco-Johnson – University of Colorado Health Sciences Center, Aurora, CO; Pier Mannucci – Ospedale Maggiore di Milano, Milan, Italy; Prasad Mathew – University of New Mexico, Albuquerque, NM; Michal Matysiak – Department of Pediatric Hematology and Oncology, Warsaw Medical University, Warsaw, Poland; Paul D. Mintz – University of Virginia, Charlottesville, VA; Anne Neff – Vanderbilt University Medical Center, Nashville, TN; Claude Negrier – Hôpital Edouard Herriot, Centre de traitement des Hémophiles, Lyon, France; László Nemes – State Health Center, Budapest, Hungary; Kathelijne Peerlinck – Universitair Ziekenhuis Gasthuisberg, Leuven, Belgium; Claire Philipp – UMDNJ Robert Wood Johnson Medical School, New Brunswick, NJ; Steve W. Pipe – University of Michigan Medical Center, Ann Arbor, MI; Michael Recht – Phoenix Children's Hospital, Phoenix, AZ; Bruce Ritchie – University of Alberta, Edmonton, Alberta, Canada; Tadeusz Robak – Wojewodzki Specialistic Hospital, Lodz Poland; C. Rothschild, Hopital Necker Enfants – Malades, Paris, France; Ajovi Scott-Emuakpor – Michigan State University, East Lansing, MI; Mark Smith – Canterbury District Health Board, Christchurch, New Zealand; Arthur Thompson -Puget Sound Blood Center, Seattle, WA; Mario Von Depka – Medical University Hannover, Hannover, Germany; Gilbert C. White – University North Carolina at Chapel Hill, Chapel Hill, NC. We also thank Martin Lee for his valuable work in the application of the Bayesian statistical approach for inhibitor safety, and Sharon Sullivan for assistance with this manuscript.

Disclosures

M. Recht receives research support from Wyeth. J. Lusher, M. Smith and P. Mannucci are consultants for Wyeth. A. O'Brien, B. Hayward, C. Udata, D. A. Roth and S. Arkin are Wyeth employees. L. Nemes has acted as a paid consultant to Wyeth Pharmaceuticals and has received fees for speaking. C. Hay has acted as a paid speaker at symposia organized by Wyeth and has been paid to give advice in consulting panels. T. Abshire was a consultant (advisory board member) for Wyeth from 1999 to 2005. The other authors stated that they had no interests which might be perceived as posing a conflict or bias.

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