

Reformulated BENEFIX[®]: efficacy and safety in previously treated patients with moderately severe to severe haemophilia B

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Summary. BENEFIX[®], the only recombinant factor IX (FIX), has been reformulated. The reformulation involves a change in diluent and allows for more concentrated infusions of recombinant FIX. A double-blind, randomized, pharmacokinetic (PK) crossover study demonstrated that reformulated BENEFIX was bioequivalent to original BENEFIX and follow-up PK evaluation after 6 months of treatment demonstrated the PK stability of reformulated BENEFIX after multiple exposures. Favourable efficacy and safety profiles, consistent with those already well-established for original BENEFIX, were observed: 81.1% of haemorrhages resolved with only a single infusion; 85.3% of initial treatment response ratings were Excellent or Good; more than half of the subjects using reformulated BENEFIX for routine prophylaxis (11 of 17, 64.7%) had no spontaneous haemorrhages during their 6–12 month course of prophylactic treatment, with an overall spontaneous bleeding rate of 0.72 year⁻¹; and for the single surgical procedure

(knee washing), treatment was rated Useful. In addition, there was no FIX inhibitor development, allergic-type manifestations, or thrombotic complications with more than 1100 infusions (nearly 5.2 million IUs) administered in this trial. All efficacy and safety outcomes from this study were achieved with more concentrated recombinant protein infusions than that possible with original BENEFIX, and utilization of the 2000 IU per vial dosage strength, newly introduced with the reformulated product, was high (>62%). The reformulation of BENEFIX allows smaller delivery volumes and an increased choice of dosage strengths without altering the PK properties (including incremental recovery and half-life) or the established efficacy and safety profile of recombinant FIX.

Keywords: BENEFIX, factor IX, haemophilia B, pharmacokinetics

Introduction

BENEFIX[®] is the only recombinant factor IX (FIX) product for haemophilia B treatment [1]. While more stringent blood screening and purification processes have rendered high-purity plasma-derived clotting factor concentrates that are considered to be free of known viruses such as human immunodeficiency virus (HIV) and hepatitis B and C, concerns about

contamination with unknown or non-lipid-enveloped infectious agents still remain as current inactivation/removal step(s) are predominately limited to lipid-coated pathogens [1,2]. No animal-sourced or human plasma-derived proteins are used during the manufacture or formulation of BENEFIX. Thus, BENEFIX is inherently free from the risk of transmission of human-blood borne pathogens. This characteristic, coupled with the multitiered viral safety programme during manufacture (i.e., multiple chromatographic purifications and a final viral filtration step), are especially noteworthy in light of recent information suggesting that the acquired prion-associated disease, variant Creutzfeldt-Jakob disease, can be transmitted by blood [3–7].

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The well-characterized safety and efficacy of BENEFIX for haemophilia B treatment were first demonstrated in a clinical trial with previously treated patients (PTPs) [8], supplemented by reports in the surgical setting and in previously untreated patients [9,10]. Since the time of its original licensure in 1997, postmarketing studies and surveillance have further characterized BENEFIX as a safe and effective product for treatment of haemophilia B, with approximately 6500 patients worldwide estimated to have used BENEFIX. During the course of postmarketing studies and surveillance, a benign phenomenon, characterized by the appearance of agglutinated red blood cells (RBC) in the i.v. tubing, was reported during infusions when the product was mixed with blood in the administration tubing and/or syringe. Although fewer than 35 cases, in tens of thousands of infusions, have been reported, and although no clinical sequelae have been associated with the observed agglutination, efforts were undertaken to study the aetiology of, and, if possible, ultimately minimize the phenomenon. Preclinical studies showed that the agglutination was associated with the low ionic strength of the formulation buffer, and not the recombinant FIX protein itself. Thus, BENEFIX has been reformulated to increase ionic strength but still retain iso-osmolality; this was achieved with a simple change in diluent, from sterile water to 0.234% NaCl.

The reformulation also allowed for other improvements, which are anticipated to increase convenience for patients. Development of more convenient treatment options is an unrelenting priority in haemophilia care. Patients commonly infuse multiple times each week over the course of a lifetime. Thus, strategies to simplify reconstitution of the drug and minimize infusion time have been pursued. With the reformulation, more concentrated recombinant FIX protein preparations are now possible, and smaller infusion volumes can be used for higher dosing strengths. Consequently, relative to the highest available dosage strength of the parent product, up to fourfold higher concentrations of the recombinant FIX protein can be administered in one quarter of the reconstitution volume. Improvements to the product reconstitution process were also developed concurrent with the reformulation; the process now involves a needle-less reconstitution device, a pre-filled diluent syringe, and the same dilution volume for all dosage strengths, including a new 2000 IU per vial dosage strength.

Herein, we report the bioequivalence of BENEFIX in its original formulation, reconstituted in sterile water for injection (SWFI), with reformulated BENEFIX

following reconstitution in 0.234% sodium chloride solution. We also report on the safety and efficacy of reformulated BENEFIX for treatment and prevention of haemorrhages in PTPs with moderately severe to severe haemophilia B.

Subjects and methods

Study design

The study protocol was approved by the institutional review board or ethics committee at each participating site, and was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. This study involved three pharmacokinetic (PK) assessments and safety and efficacy evaluations of the reformulated BENEFIX[®] (Wyeth, Cambridge, MA, USA) product for usual haemophilia B care. Two PK assessments were conducted at study start to evaluate the bioequivalence of the original and reformulated BENEFIX[®] products using a double-blind, randomized, crossover study design. Subjects were randomized to receive a single 75 IU kg⁻¹ dosage of BENEFIX, reconstituted in SWFI, or reformulated BENEFIX, reconstituted in 0.234% sodium chloride solution. At the time of this (and all other) PK infusion(s), subjects were in a non-bleeding state and had not received any FIX replacement product during the preceding five or more days. Blood samples for FIX activity determinations were collected before and at 0.25, 0.5, 1, 3, 6, 9, 24, 36, 48, 60 and 72 h after the PK infusion. After a minimal 5-day washout, subjects then received the alternate treatment, at a dose and with blood collections identical to the first PK infusion. Open-label treatment with the reformulated product then commenced. Subjects used reformulated BENEFIX, as prescribed by the investigator, for treatment of bleeding episodes and/or for prophylaxis (intermittent, routine and/or surgery-related) to accrue at least 30 exposure days over a 6-month period (exposure day = any calendar day on which reformulated BENEFIX was received). The third and final PK assessment was open-label and conducted at the completion of this 6-month open-label treatment to evaluate the stability of reformulated BENEFIX PK after repeated exposure. Subjects again received a single 75 IU kg⁻¹ dose of reformulated BENEFIX (same lot as the initial PK assessment), and provided blood samples at the same collection times as the initial PK treatment. Subjects resumed open-label treatment if fewer than 30 exposure days had been achieved over the 6-month period with the total open-label treatment period not exceeding 12 months. Routine clinical and laboratory

evaluations were performed every 3 months during the 6–12 months study course.

Subjects

Eligible participants were at least 12 years of age, had moderately severe to severe haemophilia B (FIX activity $\leq 2\%$), had previously received treatment with a FIX replacement product(s) (≥ 150 exposure days), and had neither history nor current evidence of a FIX inhibitor (current evidence = a screening result of ≥ 0.6 BU by Bethesda inhibitor assay). Subjects had adequate hepatic, renal and immune functions (alanine and aspartate aminotransferases, or total bilirubin ≤ 2.5 times the upper limit of normal (ULN); serum creatinine ≤ 1.25 times ULN; absolute CD4 $> 400 \mu\text{L}^{-1}$ respectively), and had prothrombin times ≤ 1.25 times ULN and platelet counts $> 100\,000 \text{ mm}^{-3}$. Written informed consent was obtained from each individual (or legal representative) prior to study participation.

Study test article

The active moiety, human recombinant FIX, in the original and reformulated products is identical and was manufactured as previously described by cotransfection of a Chinese hamster ovary cell line (adopted for growth in medium free of any animal-sourced and human plasma-derived protein) with complementary DNAs encoding human FIX (F9 gene) [11,12]. SWFI and 0.234% NaCl solution serve as diluents for BENEFIX in its original and reformulated presentation respectively. For PK assessments, both formulations of BENEFIX were administered using 500 IU/vial preparations in volumes of 5 mL/vial. Higher potencies of 1000 IU/vial and 2000 IU/vial were used for all open-label reformulated BENEFIX treatments.

Laboratory assessments

All non-screening tests were conducted by a central laboratory. FIX activity was measured by a one-stage, activated partial thromboplastin time-based method using the Verify Reference Plasma as normal plasma reference (VRP, BioMerieux (Durham, NC, USA); calibrated against a World Health Organization standard). FIX inhibitor was measured by Bethesda inhibitor assay, with a positive inhibitor titre defined as ≥ 0.6 BU. AntiFIX antibody was measured by enzyme-linked immunosorbent assay (ELISA). For these antibody determinations, serum titres against plasma-derived (Mononine[®], ZCB Behring, King of

Prassia, PA, USA) and recombinant FIX were obtained in two independent ELISA tests each using a protein A-horseradish peroxidase conjugate and 2,2'-Azino-di (3-ethyl-benzthiazoline-6-sulfonate) as substrate for colorimetric readout.

Pharmacokinetic parameters

All FIX activities, at actual sampling times, were adjusted for corresponding predose levels and normalized for PK dose prior to calculations. The plasma FIX activity PK parameters were determined using non-compartmental analysis (WinNonlin, v 4.1, Pharsight Corporation, Mountain View, CA, USA). The PK parameter estimates included: maximum concentration (C_{max}), area under the FIX activity-time curve from time zero to the last measurable activity (AUC_t), area under the FIX activity-time curve from time zero to infinity (AUC_∞) and terminal-phase disposition half-life ($t_{1/2}$). *In vivo* recovery was calculated as the ratio between the observed (C_{max}) and theoretical maximum FIX activities (theoretical = PK dose divided by subject plasma volume, estimated using measured haematocrit and assuming a 45-mL plasma v/kg of body weight) and incremental recovery (K-value) was calculated as the observed maximum FIX activity (C_{max}) divided by the PK dose.

Efficacy assessments

The response of a haemorrhage to each reformulated BENEFIX on-demand infusion was evaluated by the subject/caregiver or investigator using a 4-point scale employed in previous studies (Excellent: dramatic response with abrupt pain relief and clear reduction in joint or haemorrhage site size; Good: pain relief or reduction in haemorrhage site size which was delayed or required additional infusion for resolution; Moderate: probable or slight beneficial response requiring several additional infusions for resolution; or No Response: no improvement) [8,10]. These assessments, and the number of reformulated BENEFIX infusions required to resolve each bleeding episode, were used to evaluate efficacy of on-demand treatments. The efficacy of reformulated BENEFIX prophylaxis treatment was assessed by the number of breakthrough bleeding episodes and the time of their occurrence, relative to the preceding reformulated BENEFIX infusion. Investigators rated the overall clinical response to reformulated BENEFIX therapy, with consideration of any adverse events and/or relevant clinical and laboratory findings, at 3-month intervals using a 5-point scale (Very Useful, Useful,

Slightly Useful, Useless, Unfavourable). Investigators (and/or surgeons) also used this 5-point scale to assess the efficacy of reformulated BENEFIX for surgical prophylaxis, providing ratings for the intra- and postoperative periods.

Safety assessments

Safety was assessed by routine clinical and laboratory evaluations, and by adverse event reporting. Haematology, serum chemistry and viral serologies [antibodies against HIV (types 1 and 2), hepatitis A, hepatitis B (surface and core antigen), hepatitis C and hepatitis B antigen] were determined at baseline and again at study completion. FIX inhibitor and antiFIX antibody assessments were conducted at baseline and were repeated at 3-month intervals thereafter. Thrombin activation markers (prothrombin fragment 1 + 2, thrombin-antithrombin and D-dimer) were measured prior to, and at 0.5, 3, 9 and 24 h after each PK infusion. Some of these assessments were conducted more frequently for surgical procedures.

Statistical methods

Descriptive statistics were used for PK, efficacy and safety data. Bioequivalence testing was performed using an analysis of variance in accordance with standard methods and guidelines for industry [13]. Ninety per cent confidence intervals (CI) for the ratio of geometric means of test and reference product PK parameters were constructed on the log scale using the two one-sided tests procedure. To conclude bioequivalence using this approach, the upper and lower bounds of the 90% CI (for primary PK parameters: $C_{\max}$, AUC_t and AUC_{∞}) must fall within the specified limits of 80–125%. This same method was also used to evaluate the stability of reformulated BENEFIX after repeated use, with $C_{\max}$, AUC_t and AUC_{∞} at month 6 and at baseline as test and reference data respectively. Pharmacokinetics of reformulated BENEFIX were also analysed by age. For this purpose, descriptive statistics were calculated for incremental recovery data (pooled baseline and month 6 K -values) for two subject age groups, ≤ 15 and > 15 years of age. The primary PK and efficacy analyses were performed on per-protocol populations. For PK analyses, this included all subjects who completed PK visits after the appropriate washout period; for efficacy analyses, this included all subjects who had accumulated at least 30 reformulated BENEFIX exposure days over a 6 to 12-month period. Subjects with a major protocol

violation were excluded from these per-protocol analyses. Safety analyses included all subjects who received at least one infusion of study drug (reformulated or original BENEFIX).

Results

Subjects

A total of 34 subjects received at least one dose of the original or reformulated BENEFIX products (including one subject who received one dose of original BENEFIX only). Baseline characteristics of the 34 treated subjects are summarized in Table 1. Twenty-five subjects completed the study, and of those who did not, six were withdrawn because of a protocol violation, two withdrew consent (one for personal reasons; one provided no reason), and one was lost to follow-up. The median duration of study participation was 32 weeks (range 0.14–60.71 weeks). In total, 1179 infusions of reformulated BENEFIX were administered for PK, on-demand, and/or prophylactic treatment for a cumulative total of 5 199 532 IU.

Pharmacokinetics, bioequivalence and stability

The mean FIX activity-vs.-time profiles of the original and reformulated products exhibited the same multiphasic disposition and were nearly identical (Fig. 1, per-protocol analysis, $n = 24$). Results from bioequivalence testing (Table 2) demonstrate the bioequivalency of the two products, as the 90% CIs about the ratios of reformulated BENEFIX to original BENEFIX geometric means of $C_{\max}$, AUC_t and AUC_{∞} were all within the required limits of 80–125%. Similar half-life and recovery estimates (with the latter also meeting bioequivalence criteria in corresponding analyses) were also found for the original and reformulated products (Table 2).

Table 1. Demographic and baseline characteristics for subjects treated with study drug ($n = 34$).

Age (years), mean (range)	28.32 (12–61)
Race n (%)	
White	24 (70.6)
Black	6 (17.6)
Asian	1 (2.9)
Other	3 (8.8)
Lifetime exposure to FIX products (days), mean $\pm$ SD	523.38 $\pm$ 472.23
Positive family history of haemophilia B, n (%)	21 (61.8)
Positive family history of FIX inhibitor, n (%)	1 (2.9)

FIX, factor IX; SD, standard deviation.

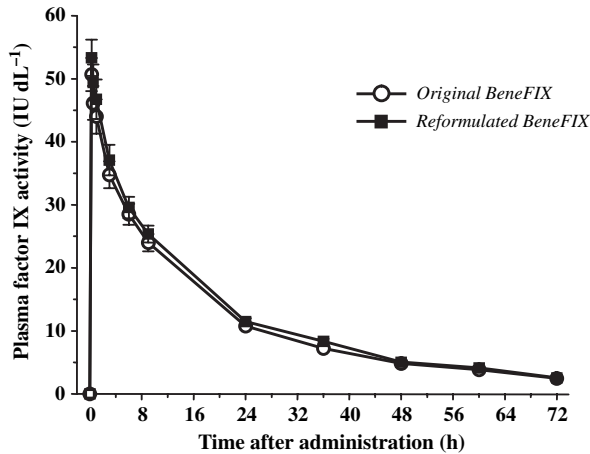


Fig. 1. Mean $\pm$ SE factor IX activity-vs.-time profiles following a 75-IU kg^{-1} infusion of original BENEFIX or reformulated BENEFIX for 24 subjects.

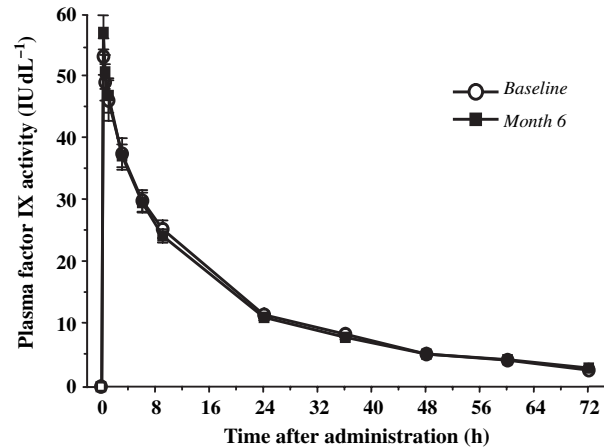


Fig. 2. Mean $\pm$ SE factor IX activity-vs.-time profiles of reformulated BENEFIX at initial exposure (75 IU kg^{-1} at baseline) and following repeated exposure (75 IU kg^{-1} at month 6) for 23 subjects.

As shown in Fig. 2 and Table 3, the PK profile of reformulated BENEFIX did not change after repeated use over a 6-month duration, as mean FIX activity-vs.-time profiles of the reformulated product at baseline and month 6 were nearly superimposable (per-protocol analysis, $n = 23$; exposure days at month 6, median = 35, range = 11–72). Furthermore, application of the same bioequivalency testing methods to these data sets produced results that met associated criteria (Table 3).

The 23 subjects with incremental recovery values following initial (baseline) and repeated (month 6 PK assessment) reformulated BENEFIX exposures were also included in the age-dependent analyses of these data. On average, lower mean incremental recoveries were found for younger subjects, ≤ 15 years of age (K -value 0.66 ± 0.16 , range $0.44\text{--}0.92\text{ IU dL}^{-1}$ per IU kg^{-1} , $n = 7$ subjects), compared with older subjects, >15 years of age (K -value 0.78 ± 0.19 , range $0.39\text{--}1.2\text{ IU dL}^{-1}$ per IU kg^{-1} , $n = 16$ subjects).

Haemostatic efficacy

The prescribed reformulated BENEFIX regimen(s) during the open-label treatment period are shown in

Table 4 ($n = 20$ subjects, per-protocol population). In summary, three subjects were prescribed on-demand regimens, exclusively; 13 subjects were prescribed routine prophylaxis regimens, exclusively; three subjects were each initially prescribed on-demand regimens, but were later switched to routine prophylaxis regimens; and one subject received reformulated BENEFIX in the context of three different treatment settings, on-demand, routine prophylaxis and surgery-related support. Prescribed routine prophylaxis therapies were supplemented with on-demand infusions to treat haemorrhages as needed. A total of 3 860 760 IU of reformulated BENEFIX were delivered by 914 infusions over 897 exposure days for open-label treatment (Table 5). Of this cumulative dose, 1 452 360 IU (37.6%) were administered using the 1000 IU/vial potency and 2 408 400 IU (62.4%) were administered using the new 2000 IU/vial potency.

A total of 95 haemorrhages were treated with 124 infusions of reformulated BENEFIX during the 6–12 month open-label period of the study ($n = 15$ subjects of per-protocol population; Table 5). Of these 95 haemorrhages, more than half were because

Table 2. Mean $\pm$ SD estimates of PK parameters for original and reformulated BENEFIX in 24 subjects.

	$C_{\max}$ (IU dL $^{-1}$)	AUC_t (h·IU dL $^{-1}$)	AUC_{∞} (h·IU dL $^{-1}$)	$t_{1/2}$ (h)	<i>In vivo</i> recovery (%)	Incremental recovery [(IU dL $^{-1}$)/(IU kg $^{-1}$)]
Original BENEFIX	51.2 $\pm$ 13.6	792 $\pm$ 192	880 $\pm$ 220	23.4 $\pm$ 5.2	32.5 $\pm$ 8.4	0.683 $\pm$ 0.182
Reformulated BENEFIX	54.5 $\pm$ 15.0	851 $\pm$ 215	940 $\pm$ 237	22.4 $\pm$ 5.3	34.5 $\pm$ 9.3	0.726 $\pm$ 0.200
Geometric mean ratio %	106	107	107		106	106
90% log-transformed CI	102–110	103–111	102–111		102–110	102–110

SD, standard deviation; PK, pharmacokinetic; $C_{\max}$, maximum concentration; AUC_t , area under the factor IX activity-time curve from time zero to the last measurable activity; AUC_{∞} , area under the factor IX activity-time curve from time zero to infinity; $t_{1/2}$, terminal-phase disposition half-life; CI, confidence interval.

Table 3. Mean $\pm$ SD estimates of PK parameters for reformulated BENEFIX at baseline and month 6 in 23 subjects.

	$C_{\max}$ (IU dL ⁻¹)	AUC_t (h·IU dL ⁻¹)	AUC_{∞} (h·IU dL ⁻¹)	$t_{1/2}$ (h)	<i>In vivo</i> recovery (%)	Incremental recovery [(IU dL ⁻¹)/(IU kg ⁻¹)]
Baseline	54.0 $\pm$ 15.2	843 $\pm$ 225	927 $\pm$ 247	21.9 $\pm$ 5.3	34.3 $\pm$ 9.1	0.720 $\pm$ 0.203
Month 6	57.3 $\pm$ 13.2	824 $\pm$ 177	923 $\pm$ 205	23.8 $\pm$ 6.5	36.8 $\pm$ 8.7	0.764 $\pm$ 0.176
Geometric mean ratio %	107	98.9	101		108	107
90% log-transformed CI	100–115	92.8–105	93.5–108		101–116	100–115

SD, standard deviation; PK, pharmacokinetic; $C_{\max}$, maximum concentration; AUC_t , area under the factor IX activity-time curve from time zero to the last measurable activity; AUC_{∞} , area under the factor IX activity-time curve from time zero to infinity; $t_{1/2}$, terminal-phase disposition half-life; CI, confidence interval.

Table 4. Summary of prescribed reformulated BENEFIX regimen(s).

	Duration on prescribed regimen (weeks)*		
	On-Demand [†]	Surgery [†]	Routine prophylaxis [‡]
<i>Subject</i> [‡]			
000101	50.6		
000136	39.0		
000139	41.1		
000116			40.0
000117			27.1
000118			25.0
000119			24.9
000124			23.9
000127			21.9
000146			26.1
000152			37.7
000153			25.6
000157			34.4
000201			25.1
000206			26.0
000208			24.0
000126	3.0		18.9
000207	13.0 [§]		22.3
000209	0.1		23.9
000141	17.9	2.9	9.3

*For subjects prescribed >1 regimen during the open-label treatment period, the regimen sequence in this table reflects actual chronological order of prescriptions.

[†]Shading indicates that no haemorrhages occurred during this time.

[‡]Per-protocol population ($n = 20$): subjects with no major protocol violations and with ≥ 30 exposure days over 6–12 months.

[§]Subject 000207 was initially prescribed on-demand treatment (2.7 weeks), then prescribed routine prophylaxis treatment (22.3 weeks), then switched back to an on-demand regimen (10.3 weeks) for the reason reported as 'personal decision'.

of injury (57.9%; remainder, 41.1% spontaneous; 1.1% origin unknown), and most occurred in a joint (50.5%) or soft tissue/muscle (37.9%) site (remainder, 10.5% multiple-site; 1.1% haematuria). All bleeding episodes were controlled exclusively with study product. Of all on-demand reformulated BENEFIX infusions (first and follow-up infusions,

$n = 124$), the median administered dose was 87.4 IU kg⁻¹, with dosages ranging from 30.3 to 147.2 IU kg⁻¹ (Table 5). Over 80% of haemorrhages (77 of 95, 81.1%) were resolved with a single infusion of reformulated BENEFIX (Fig. 3), with most joint (83.3%), soft tissue/muscle (80.6%) and multiple-site haemorrhages (70%) requiring only a single reformulated BENEFIX infusion. For those haemorrhages requiring more than one infusion, the majority were managed with two infusions (11 of 18; 61.1%). Treatment response to the first reformulated BENEFIX infusion was rated Excellent or Good for 85.3% of haemorrhages, and ratings were associated with haemorrhages occurring at each location site, with most joint (85.4%), soft tissue/muscle (86.1%) and multiple-site (90%) haemorrhages having an Excellent or Good response to initial treatment. No ratings of No Response were reported (Fig. 3, inset). A wide range of doses was used to initiate treatment of bleeding episodes (>30 to >120 IU kg⁻¹), with comparable numbers of infusions delivering between >30 to ≤ 60 (30 of 95, 31.6%), >60 to ≤ 90 (23 of 95, 24.2%), or >90 to ≤ 120 IU kg⁻¹ (38 of 95, 40.0%) and only three (3.2%) infusions delivered >120 IU kg⁻¹ (one dose not reported). Thus, the high percentage of haemorrhages requiring only a single infusion for resolution and the high percentage of Excellent or Good response ratings were not simply a consequence of higher doses. Similarly, these favourable outcomes were not limited to a particular haemorrhage type, with respect to anatomical site, but instead were significant for all types.

A total of 17 subjects (per-protocol population) used reformulated BENEFIX for routine prophylaxis during the open-label period of the study (mean duration of routine prophylaxis treatment, 25.6 weeks; range 9.3–40.0 weeks). During investigator-prescribed prophylaxis regimens, six subjects (35.3%) experienced no bleeding episodes while 11 subjects reported a total of 26 haemorrhages (27.4% of the 95 total haemorrhages reported). As shown in Fig. 4, most haemorrhages reported in the context of

Table 5. Summary of reformulated BENEFIX dosing and exposure days for open-label treatment*.

Per-protocol population (<i>n</i> = 20)*	Reason for infusions†		
	On-demand/Follow-up	Prophylaxis‡	Surgery related
Number of subjects	15	20‡	1
Total units (IU) per subject			
Cumulative	628 140	3 120 160	112 460
Median	22 380	115 150	112 460
Range	3920–166 760	58 800–399 240	112 460–112 460
Dose (IU kg ⁻¹) per infusion			
Median	87.4	51.7	34.6
Mean ± SD	77.9 ± 32.3	60.4 ± 28.2	35.7 ± 10.8
Range	30.3–147.2	13.9–184.2	25.4–57.6
Number of infusions per subject			
Cumulative total	124	753	37
Median	5	38	37
Mean ± SD	8.3 ± 7.8	37.7 ± 13.8	37
Range	1–27	15–67	37–37
Exposure days per subject			
Cumulative total	124	753	21
Median	5	38	21
Mean ± SD	8.3 ± 7.8	37.7 ± 13.8	21
Range	1–27	15–67	21–21

*Per-protocol population (*n* = 20): subjects with no major protocol violations and with ≥30 exposure days over 6–12 months.

†A subject may be dosed more than once a day for different reasons.

‡Prophylaxis includes routine (692 infusions) and intermittent (61 infusions) prophylaxis treatments. Seventeen subjects practiced routine prophylaxis; three subjects, not practicing routine prophylaxis, administered reformulated BENEFIX for intermittent prophylaxis.

SD, standard deviation.

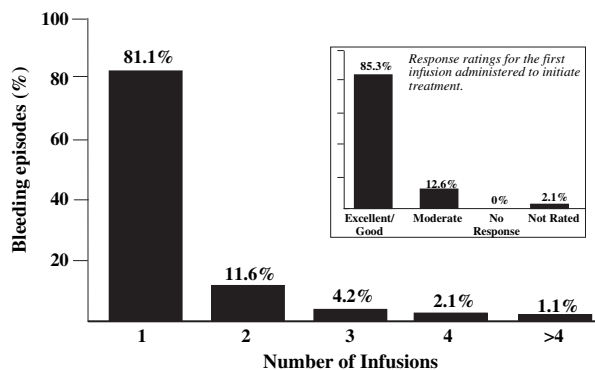


Fig. 3. Number of reformulated BENEFIX infusions required for resolution of 95 haemorrhages, and (inset) response ratings for the first infusion of reformulated BENEFIX administered to initiate treatment of 95 haemorrhages.

routine prophylaxis were caused by injury (73.1%, 19 of 26), with only six haemorrhages (23.1%) being spontaneous in nature (one haemorrhage origin unknown). In addition, most haemorrhages occurred >48 h after the last reformulated BENEFIX infusion (73.1%, 19 of 26; Fig. 4). For haemorrhages occurring within 48 h of a prophylactic infusion, only two

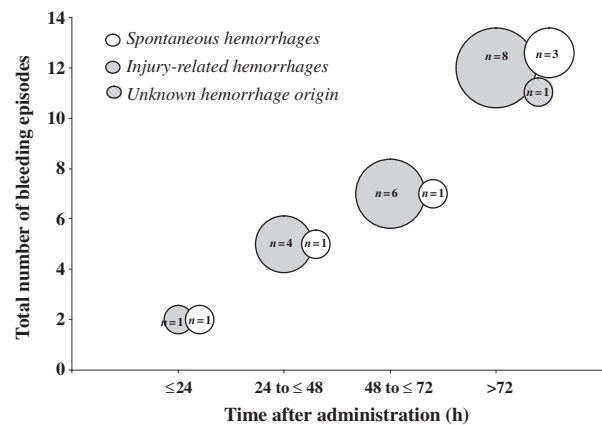


Fig. 4. Number of spontaneous and injury-related haemorrhages experienced during reformulated BENEFIX routine prophylaxis, and the time of their occurrence relative to the prior reformulated BENEFIX infusion.

were spontaneous in nature, including a joint haemorrhage reported by one subject who experienced a total of two spontaneous bleeding episodes during prophylaxis treatment and haematuria reported by one subject who experienced no other haemorrhages during prophylaxis therapy. The event of haematuria

at 47.5 h following a routine prophylaxis dose was the only early breakthrough haemorrhage classified per protocol as 'lack of effect,' as it occurred within 48 h of a prophylactic dose and with no confounding factors to explain occurrence of the episode. The six spontaneous haemorrhages that occurred during routine prophylaxis treatment were reported by six subjects; thus more than half of subjects using reformulated BENEFIX for routine prophylaxis (11 of 17, 64.7%) had no spontaneous bleeding episodes during their 6–12 month-course of prophylactic treatment. Spontaneous and total annualized bleeding rates were 0.72 and 3.11 year⁻¹ respectively. These rates were associated with multiple, subject-specific, prophylaxis regimens, with the majority of subjects on a prescribed schedule of two infusions per week.

As noted, a two infusion per week schedule was the most commonly prescribed dosing frequency (10 of 17 subjects, 58.8%). For the remainder, a nearly equal divide between <2 and >2 infusion per week schedules were prescribed (one infusion per week was prescribed for three subjects, while 2.5, 3 and >3 infusions per week were prescribed for one, one and two subjects respectively). Prescribed-dosages for reformulated BENEFIX prophylactic infusions ranged from 20 to <130 IU kg⁻¹, and were likewise variable within each dosing frequency category. For these 17 subjects, 13 practiced routine prophylaxis for the entire duration of the open-label treatment period with no prescribed regimen changes (i.e., no change in infusion frequency or dosage). The remaining four subjects initially practiced on-demand treatment with reformulated BENEFIX, but later switched to a single prescribed regimen that was unchanged for the duration of their prophylaxis therapy (Table 4). In total, 753 infusions of reformulated BENEFIX were administered for prophylaxis (692 routine, 61 intermittent) using a median dose of 51.7 IU kg⁻¹ (range 13.9–184.2 IU kg⁻¹; Table 5).

During the open-label period, there was one surgical procedure, during which the subject received reformulated BENEFIX for support during a surgical wash of the left knee. For this purpose, 37 bolus infusions of reformulated BENEFIX were administered over 2.9 weeks (21 exposure days) at a median dose of 34.6 IU kg⁻¹ (range 25.4–57.6 IU kg⁻¹; Table 5). No transfusions were required.

Investigator 5-point global assessments of reformulated BENEFIX for usual treatment of haemophilia B were considered Very Useful or Useful for 99% of reported assessments (66 of 67 visits, Fig. 5). For the single surgical case, the investigator's overall assess-

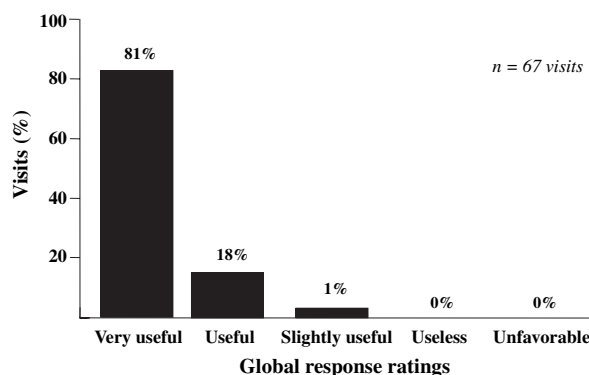


Fig. 5. Investigator assessments of reformulated BENEFIX treatment.

ment of intra- and postoperative reformulated BENEFIX treatment was Useful.

Safety

In total, 5 199 532 IU of reformulated BENEFIX, delivered by 1179 infusions over 1161 exposure days, were administered in this study for PK and open-label treatments ($n = 34$ subjects; including all subjects who received one or more infusion(s) of study product, reformulated or original BENEFIX). Of this cumulative dose, 331 892 IU (6.4%) were administered using the 500 IU/vial potency (PK assessments only), 1 809 080 IU (34.8 %) were administered using the 1000 IU/vial potency and 3 058 560 IU (58.8%) were administered using the new 2000 IU/vial potency.

There was no reports of FIX inhibitors, allergic-type manifestations, or thrombosis during the study. All ELISA results were negative for the presence of antiFIX antibody for all subjects throughout the study period. No postbaseline changes in clinical laboratory results, vital signs, or physical examinations were clinically significant. There were no serious adverse events (SAEs) related to reformulated BENEFIX. Two subjects reported unrelated SAEs. One SAE was cellulitis, the other SAE was pyogenic arthritis (treated with surgical wash) and both events resolved. One subject reported an unrelated, serious haemophilia-event (an adverse event related to the condition of haemophilia) of haematuria that also resolved. Three subjects experienced treatment-emergent adverse events that were at least possibly related, all of mild severity, which included headache, nausea, dizziness and unpleasant taste. There were no reports of viral disease transmission related to reformulated BENEFIX use (one subject, with a negative result for antibody to hepatitis A virus at

study entry, had a positive result at study completion; but follow-up testing was negative, consistent with the prior result being a false positive). One subject, with a history of RBC agglutination coincident with administrations of original BENEFIX, reported two instances with the reformulated product. These two instances, with no associated adverse events, were observed for the subject's first and second open-label treatments. For this subject, 38 subsequent infusions were administered without recurrence of RBC agglutination.

Discussion

The direct PK comparative assessment conducted in this study demonstrated that the reformulated product was bioequivalent to the original product, supporting the conclusion that reformulated BENEFIX should exhibit the same favourable therapeutic profile as the original product [8–10,14]. The predicted therapeutic equivalency was confirmed in the open-label, haemophilia B care setting of this trial. Similar to the historical data for original BENEFIX [8], most haemorrhages required only a single infusion of reformulated BENEFIX for resolution (81% vs. 81.1% for original and reformulated BENEFIX respectively), haemorrhage response to initial on-demand treatment was Excellent or Good for most episodes (91% vs. 85.3% for original and reformulated BENEFIX, respectively), and bleeding episodes were well managed by routine prophylaxis as breakthrough haemorrhage frequency was low overall and tended to correlate with the time from last prophylactic infusion (79.3% and 73.1% of haemorrhages occurring more than 48 h after the last infusion for original and reformulated BENEFIX respectively). Efficacy of reformulated BENEFIX was also shown in one case in the surgical setting. The results of this experience are consistent with those reported in the original BENEFIX studies.

While similarly favourable efficacy data for the original and reformulated BENEFIX products were achieved using a comparable range of doses (on-demand: 6.5–224.6 IU kg⁻¹ and 30.3–147.2 IU kg⁻¹; prophylaxis: 9.7–170.6 IU kg⁻¹ and 13.9–184.2 IU kg⁻¹, for original and reformulated BENEFIX respectively), corresponding median doses were higher for the reformulated product study (on-demand: 42.8 and 87.4 IU kg⁻¹; prophylaxis: 35.1 and 51.7 IU kg⁻¹, for original and reformulated BENEFIX respectively) [8]. Because these data were not derived from a single, direct comparative study, aspects related to the subject populations and study designs may have contributed to the higher median

doses used in this study. For example, the original BENEFIX studies included subjects with milder forms of haemophilia B (FIX activity up to <5%), compared with subjects in this study (FIX activity up to ≤2%); this could have accounted for the selection of doses. Most importantly, no dose escalations were prescribed during the open-label treatment period in this study, indicating that prescribed dosing reflected, and effectively met, the individual clinical needs of the subjects in this study.

Each of the following findings suggest favourable clinical response to reformulated BENEFIX treatment in this trial: the absence of any 'No Response' ratings to reformulated BENEFIX on-demand treatments; the result that most subjects (64.7%) experienced no spontaneous bleeding episodes while administering routine prophylaxis with reformulated BENEFIX; and the low-bleeding rates observed during reformulated BENEFIX routine prophylaxis therapy (0.72 spontaneous haemorrhages per year, 3.11 total haemorrhages per year). Although this study was not designed for a direct comparison of treatment regimens, the bleeding rates associated with routine prophylaxis are notably lower than the corresponding rates during periods when prescribed treatment was limited to on-demand infusions only (10.45 spontaneous haemorrhages per year, 21.84 total haemorrhages per year). Notably lower bleeding rates were also found at the individual subject level, with subjects, who switched between on-demand and routine prophylaxis regimens showing reduced bleeding rates during prophylaxis treatment [a >75% reduction in annualized bleeding rate was found for each subject (000126, 000141, 000207); this calculation was not performed for subject 000209 due to the limited duration of on-demand therapy].

The results summarized above, and the favourable global assessments for reformulated BENEFIX treatment (with most ratings of Very Useful or Useful), demonstrate the clinical efficacy of reformulated BENEFIX for usual haemophilia B care. In addition, the nearly identical PK profiles of reformulated BENEFIX after initial and repeated exposures show the stability of the reformulated product over time, consistent with the persistence of reformulated BENEFIX clinical efficacy observed for the duration of this study.

The safety data for reformulated BENEFIX were consistent with the well-established safety profile of original BENEFIX [8–10]. No SAEs related to reformulated BENEFIX occurred and the incidence of treatment-related adverse events was low. There was no report of FIX inhibitor development, no

detection of antiFIX antibody generation, no occurrence of allergic-type manifestations, no thrombogenic events and no evidence of product related-viral transmission. There were two occurrences of RBC agglutination concurrent with reformulated BENEFIX infusions reported by one subject and, like all other known instances of the phenomenon, these occurred without any clinical sequelae. This subject, with a history significant for recurrent RBC agglutination with original BENEFIX, may be predisposed to the phenomenon. Preclinical studies support the notion of an inherent predisposition for RBC agglutination as saline alone (154 mM NaCl) induced the event in approximately 15% of individual blood samples from healthy volunteers (Wyeth data on file). This observed spontaneous agglutination led to the expectation that the reformulation would reduce but not eliminate instances of RBC agglutination. Most importantly, all data on RBC agglutination continue to confirm that the phenomenon is not associated with any clinical sequelae.

This comprehensive evaluation demonstrates the therapeutic equivalence of reformulated BENEFIX and original BENEFIX. The reformulation altered neither the PK properties of the recombinant FIX protein nor the clinical efficacy and safety of the product. In addition, the more concentrated recombinant FIX protein infusions delivered with reformulated BENEFIX defined a favourable therapeutic profile for the reformulated product, consistent with that established for original BENEFIX [8–10]. All clinical efficacy and safety outcomes reported in this study were achieved using infusions that were two to fourfold more concentrated than that possible with original BENEFIX. During the open-label segment of the study, nearly 5 million IU of reformulated BENEFIX was administered for haemorrhage cessation, prophylaxis and/or surgery and more than half (62.8%) of these administrations utilized the new 2000 IU dosage strength. In light of this dosing information, the absence of FIX inhibitor development, allergic-type manifestations, or thrombogenic complications is especially important, demonstrating that manufacturing changes to reformulate BENEFIX have not altered the favourable safety profile of the product. Ongoing surveillance, currently in place for original BENEFIX, will continue to be important for the reformulated product. Pharmacokinetic results in this study also reaffirm the continuing need to monitor the PK of FIX replacement products over time. Age-dependent differences in the PK properties of both original BENEFIX and of plasma-derived FIX products have been reported [8,10,15–17]. Consistent with these

reports, a trend toward lower recoveries in younger subjects (≤ 15 vs. >15 years) was likewise observed for reformulated BENEFIX. Thus, monitoring PK over time will be important to titrate doses accordingly, and thereby ensure proper administrations of reformulated BENEFIX.

Conclusions

BENEFIX, a pathogen-free source of FIX, has been reformulated and the reformulated product is effective and safe for treatment of haemophilia B. In addition, the reformulated product offers important new features: a simplified reconstitution process, increased choice of dosage strengths and reduced delivery volumes, possibly allowing for decreased administration times. All of these new features are expected to improve convenience for patients.

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