



ORIGINAL ARTICLE *Inhibitors and allergic reactions*

A retrospective study to describe the incidence of moderate to severe allergic reactions to factor IX in subjects with haemophilia B

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Summary. Using a patient chart review process, we conducted a retrospective study to describe the frequency of allergic reactions in individuals with haemophilia B receiving factor IX (FIX) replacement therapy. The number of allergic reactions in individuals receiving a recombinant FIX (rFIX) product (BENEFIX[®]) was then compared with the number of reactions in patients receiving plasma-derived FIX (pdFIX) products. Of the 180 subjects in the study, 163 received rFIX, 88 received pdFIX; 71 received both product types. A total of seven (3.89%) subjects had a moderate or severe allergic reaction to a FIX product (95% confidence interval [CI], 1.06–6.71%). Among those receiving rFIX, four subjects (2.45%) had an allergic reaction (95% CI, 0.08–4.83%). Of individuals taking pdFIX products, three (3.41%) developed an allergic reaction (95% CI, 0–7.20%). It was noted that three (1.84%) of

those taking rFIX developed an inhibitor to FIX (95% CI, 0–3.90%), while four (4.55%) of those receiving a pdFIX product developed an inhibitor (95% CI, 0.19–8.90%). Inhibitor development was frequently associated with allergic reaction. These results provide evidence that there is no difference in the frequency of allergic reactions or inhibitor development in individuals receiving rFIX compared with those receiving pdFIX concentrates. The current study and a previous study of similar design have now compared the rate of allergic reactions associated with rFIX and pdFIX concentrates has now been compared in a total of 414 subjects; this represents the largest collection of data to date on this rare complication of haemophilia B therapy.

Keywords: allergic reactions, haemophilia, inhibitors, pdFIX, rFIX

Introduction

Haemophilia B is an X-linked disorder caused by a congenital deficiency of coagulation factor IX (FIX) [1]. Subjects with haemophilia B receive intravenous replacement therapy with FIX concentrates for the control or prevention of haemorrhage. Plasma-derived FIX (pdFIX) concentrates have been available since 1990 [2]. In 1997, a recombinant FIX (rFIX) product, BENEFIX[®] (Wyeth, Cambridge, MA, USA), was licensed

in the United States and Europe for the prevention and control of haemorrhagic episodes and intraoperative bleeding in subjects with haemophilia B.

Potentially serious—though uncommon—complications of FIX replacement therapy include severe allergic reactions to FIX and development of FIX-neutralizing antibodies [3]. Neutralizing antibodies (inhibitors) usually occur in subjects with major derangements of the FIX gene, such as large deletions, stop codons and frameshift mutations. The risk for inhibitor development may be as high as 50% in subjects with total gene deletions and up to 20% in those with frameshift mutations and stop codon abnormalities. Smaller missense mutations do not appear to be associated with any increase in inhibitor formation [3–5].

Anaphylaxis is a serious complication of FIX infusions, particularly in individuals with a history of

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Accepted after revision 3 September 2010

developing FIX inhibitors [3,6]. Inhibitors may or may not be associated with other, less severe systemic allergic reactions, and inhibitor development can precede, occur simultaneously with or occur after an allergic reaction to FIX [3].

To gain additional insight into the occurrence of allergic reactions and the link between such reactions and inhibitor development in individuals being treated for FIX deficiency, we conducted a retrospective study of subjects with haemophilia B receiving replacement therapy with pdFIX or rFIX. This study was developed as a postregistration regulatory commitment to supplement data from a previous study of allergic reactions in subjects with haemophilia B treated with FIX (Wyeth Pharmaceuticals, Data S1).

Methods

Study design

This study used a retrospective, multicentre design and was conducted in subjects with FIX deficiency at centres in North America and Europe. It involved a medical chart review of eligible subjects; only demographic, treatment and safety data were collected. No factor concentrate was provided to subjects, and no prospective interventional procedures were performed. The primary objective of the study was to estimate the frequency of moderate or severe allergic reactions associated with the administration of any FIX replacement therapy. Secondary objectives were: (i) to compare the frequency of moderate or severe allergic reactions in subjects receiving pdFIX with the frequency of such reactions in subjects receiving rFIX and (ii) to evaluate the occurrence of inhibitor formation in subjects affected by moderate or severe allergic reactions.

The choice of product and infusion frequency for this study were determined by the treating physician. All centres in the European Union (EU) that were contacted with a request to participate in the BENEFIX EU Registry (a separate safety registry) were contacted and invited to participate in this study. In addition, haemophilia treatment centres in North America that treat more than 15 patients with haemophilia B and did not participate in a previous retrospective study of allergic reactions with FIX concentrates were requested to participate in the current study. Informed consent was obtained from each subject (or from the appropriate legally authorized representative), and eligibility was established by chart review.

Subjects who satisfied the following inclusion criteria were eligible to participate in this study: (i) written consent to release information on the subject, (ii) diagnosis of mild to severe haemophilia B (FIX:C $\leq$ 25%) and at least one exposure to FIX replacement product(s), and (iii) receipt of the first infusion of any FIX product between 1 January 1991 and 31 December 2003.

Safety measurements

Because this study was designed to assess the incidence of moderate to severe allergic reactions, data were obtained from subject charts whenever a class II or class III allergic reaction had been reported within 24 h of administration of any FIX product. For this study, a class II reaction was defined as a moderate allergic event with respiratory involvement, which included dyspnoea and hypoxia, as well as general symptoms of hypersensitivity (cough and chest tightness). A class III reaction was defined as an allergic event characterized by severe anaphylaxis with hypotension, including anaphylactic reactions, anaphylactoid reactions, anaphylactic shock, bradycardia, bronchospasm, cyanosis, hypotension, laryngeal oedema, laryngospasm and syncope. Class I reactions, which are mild allergic reactions limited to dermal involvement, were not the focus of this study.

In addition to demographic, treatment and safety data, detailed allergic reaction data were collected from charts of subjects reported to have experienced a class II or class III allergic reaction within 24 h of receiving a FIX product. The chart of each subject who developed a FIX inhibitor or who experienced a class II or class III allergic reaction was reviewed to assign causality of the event to past rFIX or pdFIX use. Information on FIX inhibitor development [$\geq$ 0.6 Bethesda Units (BU)] was collected from patient charts regardless of its temporal relation to onset of allergic reaction. The assignment of an allergic event to prior FIX use was based on the recorded dates of FIX exposure, their timing relative to the occurrence of an allergic event and the clinical judgment of the investigator.

The types of allergic symptoms, as well as the medications used to treat the allergic reactions, were also recorded. The presence or absence of fever or other recent illnesses (within 7 days of infusion), recent immunizations (within 30 days of infusion) and concomitant medications taken within 24 h of FIX infusion were also documented. The outcomes of allergic reactions as well as the use of any additional evaluations (e.g., skin testing, radioallergosorbent test) and the results of rechallenge with FIX (if applicable) were also noted.

The following potentially relevant information was also collected from the subjects' charts: (i) exposure day history (defined as any calendar day in which a subject received at least one infusion); (ii) adverse events occurring during the 48 h after onset of a class II or class III allergic reaction in response to any product; and (iii) serious adverse events possibly, probably or definitely related to rFIX infusion. All other events of special interest that occurred during periods of rFIX treatment at any time during the retrospective study review period were also analysed. These included thrombogenicity, red blood cell agglutination and less-than-expected therapeutic effect and/or poor FIX

recovery. All data collected were checked for accuracy and completeness and were reviewed by the investigator. A second monitor at each site verified source document accuracy by comparing case report forms against subject medical charts.

Statistical analysis

All subjects enrolled in the study were considered to be evaluable. The statistical analyses were descriptive. For categorical variables, the number and percentage of each category were calculated. For continuous variables, the mean, median and standard deviation, as well as minimum and maximum values, were presented. Data from all sites were combined for each analysis. The overall frequency of allergic reactions was reported, as were indications that the reactions were associated with pdFIX or rFIX. The total frequency of reactions was calculated by dividing the number of subjects with allergic reactions associated with the administration of any FIX concentrate by the total number of subjects enrolled. Frequencies by product category were calculated by dividing the number of subjects with allergic reactions to the type of product (rFIX or pdFIX) by the total number of subjects infused with that product category. The 95% confidence interval (CI) estimates were provided for each of these incidence estimates. The overall sample size was agreed on by the European Medicines Agency and Wyeth Research.

Results

Subject characteristics

Of 180 subjects enrolled in the study, 177 were male and 154 were white (Table 1). Subjects ranged in age from 2.4 to 82.6 years (mean, 18.05 years) at the time of consent or assent. All but one subject was alive at the time of the review. A total of 43.9% of the subjects had severe haemophilia B (FIX:C <1%), 35.6% had moderate haemophilia B (FIX:C 1–5%) and 20.6% had mild haemophilia B (FIX:C >5–25%). Genotypes were known in 41.7% of the subjects. There were no clinically meaningful differences in the severity of haemophilia B or in the genotype between subjects who received rFIX and those who received pdFIX products (Table 1).

Safety

Both the extent of exposure and the product type were recorded. Of the 180 subjects in the study, 163 received rFIX, 88 received pdFIX and 71 received both rFIX and pdFIX. (Patients who received both pdFIX and rFIX during the study period were counted in both groups). Two of the 163 subjects who received rFIX also received another unspecified FIX product. Three of the 71 subjects who were initially reported to have received both rFIX and pdFIX did not actually receive pdFIX as a replacement product but instead received factor VII

Parameter	All subjects N = 180	Subjects taking rFIX n = 163	Subjects taking pdFIX n = 88	Allergic reaction n = 7	No reaction n = 173
Mean age, years	18.05	17.7	20.1	21.53	17.91
Age range, years	2.4–82.6	2.4–82.6	3.3–82.4	6.2–77.8	2.4–82.6
Gender, no. subjects (%)					
Male	177 (98.3)	160 (98.2)	86 (97.7)	7 (100.0)	170 (98.3)
Female	3 (1.7)	3 (1.8)	2 (2.3)	0 (0.0)	3 (1.7)
Race, no. subjects (%)					
Caucasian	154 (85.6)	143 (87.7)	73 (83.0)	3 (42.9)	151 (87.3)
Black	1 (0.6)	1 (0.6)	1 (1.1)	0 (0.0)	1 (0.6)
Asian	2 (1.1)	1 (0.6)	2 (2.3)	0 (0.0)	2 (1.2)
Hispanic	5 (2.8)	4 (2.5)	4 (4.6)	2 (28.6)	3 (1.7)
Other	4 (2.2)	3 (1.8)	1 (1.1)	1 (14.3)	3 (1.7)
Unknown	14 (7.8)	11 (6.8)	7 (8.0)	1 (14.3)	13 (7.5)
Family allergy history, no. subjects (%)					
Yes	24 (13.3)	22 (13.5)	15 (17.1)	3 (42.9)	21 (12.1)
No	67 (37.2)	61 (37.4)	35 (39.8)	0 (0.0)	67 (38.7)
Unknown	89 (49.4)	80 (49.1)	38 (43.2)	4 (57.1)	85 (49.1)
Subject allergy history, no. subjects (%)					
Yes	50 (27.8)	47 (28.8)	25 (28.4)	6 (85.7)	44 (25.4)
No	119 (66.1)	107 (65.6)	56 (63.6)	1 (14.3)	118 (68.2)
Unknown	11 (6.1)	9 (5.5)	7 (8.0)	0 (0.0)	11 (6.4)
Genotype of haemophilia B, no. subjects (%)					
Point mutations	62 (34.4)	53 (32.5)	35 (39.8)	2 (28.6)	60 (34.7)
Total gene deletions	13 (7.2)	10 (6.1)	8 (9.1)	3 (42.9)	10 (5.8)
Unknown	105 (58.3)	100 (61.4)	45 (51.1)	2 (28.6)	103 (59.5)
Haemophilia B classification, no. subjects (%)					
Severe (FIX:C <1%)	79 (43.9)	70 (42.9)	47 (53.4)	5 (71.4)	74 (42.8)
Moderate (FIX:C 1–5%)	64 (35.6)	60 (36.8)	32 (36.4)	2 (28.6)	62 (35.8)
Mild (FIX:C >5–25%)	37 (20.6)	33 (20.3)	9 (10.2)	0 (0.0)	37 (21.4)

Table 1. Summary of subject demographics.

Table 2. Summary of exposure days.

	All subjects	Subjects taking rFIX	Subjects taking pdFIX	Allergic reaction	No reaction
rFIX days					
N	151	151	62	4	147
Mean	118	118	192.2	54.8	119.7
SD	186.6	186.6	234.5	51.3	188.7
Minimum, maximum	1, 1197	1, 1197	1, 1197	*4, 121	1, 1197
Median	21	21	82.5	47	20
N unknown	12	12	9	1	11
pdFIX days					
N	78	62	78	5	73
Mean	109.8	102.5	109.8	88.2	111.3
SD	225.5	202.7	225.5	119.8	231.4
Minimum, maximum	1, 1335	1, 1335	1, 1335	*6, 300	1, 1335
Median	25.5	24	25.5	50	24
N unknown	10	9	10	2	8
Total days					
N	170	154	81	6	164
Mean	155.2	157	252.8	110	156.8
SD	274.2	271.9	352.5	116.8	278.3
Minimum, maximum	1, 1966	1, 1966	1, 1966	10, 327†	1, 1966
Median	28.5	28.5	55	58.5	25.5
N unknown	10	9	7	1	9

*Five of seven subjects received more than one replacement product during the course of the study. Thus, in this instance the same patient represents the minimal EDs for both rFIX and pdFIX in subjects exhibiting an allergic reaction.

†Maximum total EDs for this patient includes 27 EDs to rFIX and 300 EDs to rFVIIa; allergic reaction was attributed to rFIX.

(rFVIIa, NovoSeven®; Novo Nordisk, Bagsvaerd, Denmark).

Estimates of the number of exposure days (EDs) were collected for 170 of the 180 subjects (Table 2). Of these, the median number of EDs was 28.5 (range, 1–1966). Of the 170 subjects with known EDs, 151 received rFIX at some time during the study, for a median of 21 EDs (range, 1–1197), and 78 received pdFIX during the study, for a median of 25.5 EDs (range, 1–1335).

Table 3. Symptoms and assigned treatment causality.

Subject	Event		Symptom	Maximum inhibitor titre (BU)	Treatment causality assignment
	FIX inhibitor	Allergic reaction			
A	X	X	Dyspnoea; urticaria	17.9	rFIX
B	X		Inhibitor only	6.2	pdFIX
C		X	Somnolence; asthenia	NA	rFIX
D	X		Inhibitor only	9.0	rFIX
E	X	X	Wheezing; anaphylaxis with cyanosis	960.0	pdFIX
F	X	X	Wheezing; anaphylaxis; hypotension	40.0	Unknown (inhibitor) rFIX (allergic reaction)
G	X	X	Anaphylaxis; respiratory distress; hives	1.4	rFIX
H		X	Chest tightness; cyanosis; diaphoresis; dizziness	NA	pdFIX
I	X	X	Facial oedema; flushing; choking	19.2	pdFIX
J	X		Inhibitor only	NR	Unknown
K	X		Inhibitor only	NR	pdFIX

BU, Bethesda Units; NA, not applicable; NR, not recorded.

Charts review showed, allergic reactions to a FIX product in seven (3.89%) subjects (95% CI, 1.06–6.71%). Signs and symptoms of allergic reactions ranged in severity from moderate (class II: dyspnoea and urticaria, somnolence, asthenia, chest tightness, cyanosis, diaphoresis and dizziness) to severe (class III: wheezing, anaphylaxis, hypotension, respiratory distress and hives) to life-threatening (anaphylaxis with cyanosis and cardio-respiratory arrest) (Table 3). One subject experienced facial oedema, flushing and choking of unknown severity. All allergic responses were deemed related to FIX treatment. Most signs of an allergic response resolved, with only wheezing and bronchospasm in one subject persisting at last review. Of the seven subjects with allergic responses, one subject experienced a recurrent class II or class III reaction when rechallenged with rFIX. With respect to the genotypes of the subjects who experienced class II or III allergic reactions, point mutations were identified in two subjects (28.6%) and total deletions were found in three subjects (42.9%). FIX genotyping was not performed in the two remaining subjects with allergic reactions (Table 1).

The six subjects with class II or class III allergic reactions for whom EDs were known were treated for a median of 58.5 EDs (range, 10–327). Among these subjects, four received rFIX for a median of 47 EDs (range, 4–121) and five received pdFIX for a median of 50 EDs (range, 6–300) (Table 2).

Inhibitor formation with a cause assignable to a product type was observed in seven (3.89%) subjects receiving FIX products (95% CI, 1.06–6.71%) (Table 3). FIX inhibitors also developed in two other subjects, but the cause could not be assigned to a particular product type. Of these two subjects, one was receiving rFVIIa at the time of inhibitor development (1.5 years after stopping rFIX treatment); for the other subject, the details surrounding inhibitor development,

Table 4. Frequency of allergic reactions and inhibitor formation.

	Subjects taking rFIX <i>n</i> = 163	Subjects taking pdFIX <i>n</i> = 88	Total no. subjects <i>N</i> = 180
Allergic reaction			
Frequency, no. subjects (%)	4 (2.45)	3 (3.41)	7 (3.89)
95% CI	0.08, 4.83	0.00, 7.20	1.06, 6.71
Inhibitor formation			
Frequency, no. subjects (%)	3 (1.84)	4 (4.55)	7 (3.89)
95% CI	0.00, 3.90	0.19, 8.90	1.06, 6.71

CI, confidence interval.

including timing and replacement product exposure details, were not recorded.

With respect to product type, of the 163 subjects receiving rFIX, four (2.45%) experienced an allergic reaction (95% CI, 0.08–4.83%) and, of 88 subjects receiving pdFIX products, three (3.41%) experienced an allergic reaction (95% CI, 0–7.2%). Of those receiving rFIX, three (1.84%) developed inhibitors to FIX (95% CI, 0–3.9%); among those receiving pdFIX products, four (4.55%) developed inhibitors (95% CI, 0.19–8.90%). Not every subject who developed an inhibitor had an allergic reaction, and not every subject who had an allergic reaction developed an inhibitor. However, the two events frequently occurred together. When the two individuals who developed inhibitors as a result of an unknown cause are included, 5 of the 11 subjects in the study who experienced any event had both an allergic reaction and a FIX inhibitor (Table 3). The frequency of occurrence of inhibitor development and allergic reactions is summarized in Table 4.

Discussion

The primary aim of this study was to estimate the frequency of moderate and severe allergic reactions in individuals with haemophilia B receiving a FIX concentrate. A secondary aim was to compare the rate of allergic reactions in subjects receiving pdFIX with the rate of such reactions in those receiving rFIX. The frequencies for allergic reactions to rFIX and pdFIX observed in the study were 2.45% (95% CI, 0.08–4.83%) and 3.41% (95% CI, 0–7.20%), respectively. These data suggest that the upper range of the observed frequency of allergic reactions to rFIX and the overall frequency of allergic reactions to rFIX are similar to those of reactions to pdFIX. Of the seven subjects who experienced allergic reactions, four also developed inhibitors that could be assigned to rFIX or pdFIX.

This report corroborates data from a similar study, which was also carried out as a regulatory commitment. In that study, a review of 234 charts of haemophilia B patients was conducted. A total of 165 patients received rFIX and 160 received pdFIX; 91 of the 234 patients received both types of product. For the patients receiving rFIX, three (1.8%) experienced class II/III allergic

reactions as compared with seven (4.4%) of the patients receiving pdFIX. One patient in the study experienced class II/III allergic reactions following the administration of both rFIX and pdFIX. The 95% CIs for the incidence of allergic reactions to rFIX and pdFIX were 0.0–3.9% and 1.2–7.5%, respectively, indicating there was no evidence that the incidence of allergic reactions to rFIX was higher than that for pdFIX. Inhibitor formation in this prior study was observed in 6.9% of patients receiving pdFIX and in 1.8% of patients receiving rFIX (Wyeth, Data S1).

While the reasons for allergic reactions occurring at the time of inhibitor development in patients with haemophilia B have not been fully elucidated, a number of explanations have been proposed. FIX is a small molecule (55 kDa) that is distributed extracellularly; these properties may contribute to immunoglobulin E (IgE)-mediated hypersensitivity and a risk of anaphylaxis [7]. Another possible explanation for the association of inhibitor development with allergic reaction lies in the molecular genetic characterization of FIX deficiency [8]. It has been suggested that large FIX gene deletions confer the greatest risk for allergic reactions. At least 26% of FIX inhibitor patients with haemophilia B resulting from complete gene deletion were estimated to be at theoretical risk for such allergic reactions [9]. Although the exact mechanism is not certain, the complete absence of a FIX gene may occur with a co-deletion of neighbouring immune-response loci, resulting in a predisposition for allergic reactions. Furthermore, large deletions may make patients susceptible to allergic responses because of the inability to induce immune tolerance in the absence of any endogenous FIX protein as a result of total gene deletion [7].

Although inhibitor development does not always occur in persons with allergic reactions, several reported and published cases have linked these types of adverse events [3]. In this study, we found that inhibitors were formed in 1.84% and 4.55% of subjects receiving rFIX and pdFIX, respectively; the CIs indicate no marked differences in these rates. The observed frequencies for the development of allergic reactions to rFIX and pdFIX were 2.45% and 3.41% respectively. Thus, from this retrospective study of 180 subjects with haemophilia B receiving FIX replacement therapy, the frequency of moderate or severe allergic reactions, as well as the frequency of inhibitor development, did not significantly differ between subjects receiving rFIX and those receiving pdFIX.

Since the introduction of rFIX, from February 1997 through 10 August 2007, there have been 90 postmarketing reports of allergic reactions, including anaphylactoid reactions, in 89 subjects worldwide (Wyeth, Data S1). Reports of systemic allergic reactions were relatively evenly distributed among severity categories, with 33 class I reactions, 31 class II reactions and 25 class III reactions. Of the 90 reports of allergic

reactions, 26% occurred in patients known to have inhibitors. Notably, 40% of the class III reactions occurred with concomitant inhibitor development. Of the 67 reports of allergic reaction in subjects without inhibitors, 20 described patients with a known allergy or previous allergic reaction to pdFIX. Therefore, almost half of the reports described subjects with predisposing risk factors for the development of allergic reactions. There have been no reports of any deaths in subjects with possible systemic allergic reactions to rFIX since registration.

Based on the results presented in this article, no change in clinical care of the patient affected by FIX deficiency is recommended. In many centres, patients receiving FIX concentrate, whether plasma-derived or recombinant, are monitored in a controlled setting for up to the first 20 exposure days. This practice should be considered standard of care and does not need to be modified based on our results.

In conclusion, this retrospective analysis found that the overall frequency of allergic reaction to rFIX was similar to that for pdFIX. Furthermore, the observed incidence of inhibitor development was similar in individuals receiving pdFIX and rFIX. This suggests that the risk for allergic reaction or inhibitor develop-

ment with rFIX is not likely to be higher than the risk with pdFIX. This study, combined with a similarly designed, previously conducted study—which also found a similar incidence of allergic reactions to rFIX and pdFIX—represents the largest collection of data to date on the relative rates of allergic reaction and inhibitor development associated with rFIX and pdFIX concentrates, with a total of 414 participating subjects.

Acknowledgements

We wish to thank the patients, their families and the clinical personnel who participated in this study. We also acknowledge David Schwartz, PhD, of Accel Health for editorial assistance during manuscript preparation, and Gretchen Bondi of Peloton Advantage, LLC, for assistance with manuscript formatting prior to submission. No contribution was made to editorial content. Editorial support was funded by Wyeth Pharmaceuticals, which was acquired by Pfizer Inc in October 2009, and by Pfizer Inc.

Disclosures

This research was funded by Pfizer Inc, which also provided financial support for the preparation of this manuscript. No author received an honorarium or other form of financial support related to the development of this manuscript. At the time of this study, Dr. Neuman and Janco were employees of Pfizer Inc.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Data S1. Subject narratives for deaths, serious adverse events and withdrawals attributed to adverse events.

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Tribute to Dr. Robert Janco

We wish to acknowledge the contributions of our colleague, Robert Janco, MD, who passed away during the preparation of this manuscript. An outstanding clinician and friend of the hemophilia community, Dr. Janco had a wealth of experience in the diagnosis and treatment of adults and children with congenital bleeding disorders. After receiving his medical degree from Yale University, he served an internship at the David Grant Medical Center at Travis Air Base in California and a fellowship in pediatric hematology-oncology at the University of Iowa Hospitals

and Clinics. He became a Professor at Vanderbilt University, where he earned several teaching awards, and eventually served as Director of the Vanderbilt Hemophilia and Hemostasis-Thrombosis Clinics. During his fruitful career, he also served with the Tennessee State Hemophilia Program, co-chaired the National Prevention Program of the National Hemophilia Foundation, and sat on the Medical and Scientific Advisory Council of the National Hemophilia Foundation. He was appointed to the Presidential (Reagan) Commission on the HIV Epidemic, the Tennessee Commission on HIV, and led other local policy initiatives. Most recently, Dr. Janco served as

Disease Area Lead for Hematology at Pfizer. Dr. Janco lately retired from the US Air Force and from the Tennessee Air National Guard, in which he served as a physician, flight surgeon, and hospital commander for 32 years. He authored or coauthored more than 40 journal articles. Dr. Janco will be remembered for his unwavering commitment to improving the treatment of patients with bleeding disorders. We will miss him.

Supplemental information

Subject narratives for deaths, serious adverse events and withdrawals due to adverse events

Subject A – Inhibitor and class II/III allergic reaction

Subject A was 9 years old at the time of chart review. This Caucasian male was diagnosed with severe haemophilia B on 26 June 1997 and had a R29X (CGA >TGA in codon 29) substitution. The subject had no history of allergies; however, his family had an unspecified allergy history. The subject had a family history of haemophilia.

The subject received infusions of rFIX from 09 May 1998 to 04 January 1999 (approximately 25 EDs). Inhibitor monitoring on 04 June 1999 revealed a level at 0 BU.

On 16 December 1998, the subject developed an inhibitor of 3.5 BU that was considered by the investigator and the medical monitor to be related to rFIX treatment. Additionally, the subject developed an anaphylactoid reaction, which manifested with urticaria and dyspnea, immediately following replacement treatment with approximately 25 IU/kg. The anaphylactoid reaction was considered by the investigator to be non-serious and related to the BeneFIX treatment. The event resolved approximately 30 minutes after treatment with intravenous (IV) hydrocortisone. rFIX was continued without prophylactic allergy treatment. rFIX was increased from 25 IU to 1000 IU on demand in December 1998 due to reduced clinical response.

On 07 January 1999, the subject was treated at home for a bleeding episode in the right arm, without success. rFIX 1500 IU was administered in the hospital. Inhibitor levels of 12.6 BU (central laboratory) and 14 BU (local laboratory) were noted. A peak inhibitor

value of 17.9 BU was noted on 22 January 1999. Worsening dyspnea and urticaria recurred upon rechallenge with rFIX. Therefore, rFIX was discontinued (last dose administered on 07 January 1999), and treatment with rFVIIa was initiated on 28 January 1999, with successful treatment of bleeding episodes. The inhibitor persisted, with a gradual resolution in 2004; the subject had an inhibitor level of 0 BU at an unknown date in 2004. The investigator and medical monitor considered these events to be related to rFIX.

Subject B – Inhibitor

Subject B was 23 years old at the time of chart review. This Caucasian male was diagnosed with severe haemophilia B at an unknown date in 1994. The subject had a FIX gene deletion at positions 6379 to 6384. No information was available as to whether the subject or his family had any history of allergic reactions.

The subject received pdFIX therapies from 1995 to 1999, Immunine (Baxter) from 28 February 1995 to 24 August 1995 (7 EDs), and Berinin® HS 600 (Aventis Behring) from an unknown start date until 25 October 1999 (20 EDs). The subject's replacement therapy after October 1999 is unknown.

On 30 September 1999, the subject developed an inhibitor (3.2 BU). The maximum titer, recorded on 09 March 2000, was 6.2 BU. No other information is available regarding this event. A causality assessment from the investigator was not provided for this historic event.

Subject C – Class II/III allergic reaction

Subject C was 23 years old at the time of chart review. This Caucasian male was diagnosed with moderate FIX deficiency on 22 July 1994. No information was available on the subject's genotype. The subject had allergies/drug sensitivity reactions to amoxicillin (perspiration, rash, and pruritus) and to aspirin (bronchospasm). It is not known if his family had any allergic history. The subject had a family history of haemophilia.

The subject received pdFIX therapies from 1995 to 2000, Mononine® (CSL Behring; 6 EDs) and Aimafix® (Biosano; 24 to 29 EDs). The subject received on-demand infusions of rFIX (50 IU/kg) beginning on 07 December 1999. Treatment was ongoing through the time of the chart review, for a total of 121 EDs.

On 07 December 1999, the subject developed an allergic reaction, which manifested with moderate somnolence and moderate asthenia immediately after his initial dose of rFIX. The reactions lasted about a half hour and resolved without additional treatment when the infusion was slowed. The investigator and medical monitor assessed these events as being related to rFIX treatment. The subject was evaluated for inhibitors prior to additional rFIX therapy; this evaluation did not reveal the presence of an inhibitor. He continued on rFIX, and upon receipt of the next dose, the reaction recurred but was reported to be milder. Allergy prophylaxis was not administered prior to treatment with rFIX for this incident. The subject was not switched to an alternative product, and his clinical status was described as well with ongoing rFIX treatment.

Subject D – Inhibitor

Subject D was 9 years old at the time of chart review. This Hispanic male was diagnosed with severe FIX deficiency on 05 September 1994. The subject had complete gene deletion.

The subject was reported to have had an allergy to aspirin. The subject's family had allergies to aspirin, penicillin, and Augmentin® (amoxicillin/clavulanate; GlaxoSmithKline). This subject was the brother of 2 other study subjects: E and F. The subject received rFIX from 21 November 1998 to 04 December 1998 (13 EDs) and rFVIIa beginning on December 1998. rFVIIa therapy was listed as continuing (quantification of EDs not known).

On 26 January 1999, a FIX inhibitor (3 BU) was noted during a recovery study. The maximum titer, 9 BU, was recorded on 05 December 2001. The investigator and the medical monitor assessed this event to be related to the rFIX treatment, although during this time the subject was no longer receiving rFIX therapy.

Subject E – Inhibitor, class II/III allergic reaction and other serious adverse reactions

Subject E was 9 years old at the time of chart review. This Hispanic male was diagnosed with severe haemophilia B in January 1993. The subject's genotype history was a complete FIX gene deletion.

The subject had allergies to factor VIII inhibitor bypassing activity (FEIBA), aspirin, penicillin, amoxicillin, Augmentin® (GlaxoSmithKline), and urokinase. He had a family history of allergies to aspirin.

This subject was the brother of 2 other study subjects: D and F. The subject received pdFIX products—Konyne® from April 1993 to December 1993 (total EDs are not known) and Mononine® (CSL Behring) from 24 April 1993 to 05 January 1994 (48 EDs). On 10 June 1993, he developed a FIX inhibitor (16 BU). The maximum titer, 960 BU, was recorded on 05 June 1994. His only factor replacement prior to the event was pdFIX.

On 28 July 1993, the subject experienced an anaphylactic allergic reaction, which manifested with bronchospasm and wheezing. He also experienced constipation. During the period prior to these events, the subject was receiving acetaminophen and Benadryl® (diphenhydramine; McNeil-PPC, a division of Johnson & Johnson) as prophylaxis for allergic events. The investigator assessed the wheezing as related to pdFIX therapy; the relationship of the bronchospasm to pdFIX therapy was assessed as unknown. The investigator's assessment of the relationship of pdFIX and constipation, which resolved in July 1993, was also not known.

The bronchospasm resolved during August 1993; the resolution date for the bronchospasm was not available. These events were treated with oral senna and inhaled albuterol. The subject was also receiving oral phenobarbital (indication not specified) from 26 May 1992 until July 1993.

On 15 December 1993, the subject experienced bilateral acute otitis media, bilateral wheezing, cough, fever, nausea and vomiting. These events all resolved and were assessed by the investigator to be related to pdFIX treatment.

On 17 December 1993, the subject experienced two life-threatening events: anaphylactic allergic reaction, with onset during pdFIX infusion, and cardiopulmonary arrest. These events were assessed by the investigator as definitely related to pdFIX therapy, and treatment with pdFIX was discontinued.

These events were accompanied by seizure (mild) and cyanosis (moderate); these events were also assessed to be definitely related to pdFIX treatment. Additionally, on the same date, the subject experienced a right groin hematoma, retroperitoneal hematoma and anemia; these events were assessed by the investigator to be severe and not related to

pdFIX treatment. He also experienced mild tachycardia of unknown relationship to the treatment. Beginning on 17 December 1993, the subject received the following medications as treatment for these events: epinephrine (1:10,000), Benadryl®, cefuroxime, and albuterol. The subject also had a blood transfusion. He was rechallenged with pdFIX following premedication, and a subsequent allergic reaction did not occur.

Beginning on 18 December 1993, the subject also received lorazepam, as needed, and phenobarbital. FIX inhibitor testing was conducted on 20 December 1993, and the result was 0 BU. On 22 December 1993, the subject experienced a neck rash of moderate intensity that the investigator assessed to be related to pdFIX treatment; this event resolved on 27 December 1993 following unspecified drug treatment.

Subject F – Class II/III allergic reaction and inhibitor

Subject F was 8 years old at the time of chart review. This Hispanic male was diagnosed with severe haemophilia B on 21 November 1996. Genotyping revealed a complete gene deletion.

The subject had allergies to aspirin and FEIBA. His family had allergies to aspirin, FEIBA, penicillin, Augmentin®, and urokinase. This subject was the brother of 2 other study subjects: D and E. The subject was treated with pdFIX (Mononine®) from 23 to 29 October 1997 (6 EDs). He had no allergic reactions to the Mononine. The subject received rFIX from 31 October to 05 November 2003 (4 EDs) and rFVIIa beginning on 06 November 2003. rFVIIa therapy was listed as continuing (EDs unknown).

The subject began to experience mild allergic symptoms (rash) with rFIX and was given premedication with subsequent doses. On 05 November 1997, he experienced an

anaphylactic reaction, which required hospitalization and was considered life-threatening. The subject was treated with IV methylprednisolone 10 mg and IV diphenhydramine 10 mg. The reaction continued to develop, resulting in a severe anaphylactic reaction response with wheezing, flushed face, hives and hypotension. The subject was treated with additional IV diphenhydramine 20 mg, IV methylprednisolone 50 mg, plus inhaled albuterol; he recovered that day. Duration of the event was not reported. The subject was not rechallenged with rFIX and was subsequently treated with rFVIIa on demand, without symptoms. The medical monitor and the investigator considered the anaphylactic reaction to be related to rFIX therapy.

The subject also developed a FIX inhibitor on 06 April 1999 (while receiving rFVIIa), with a maximum titer of 40 BU (date not known). On 05 December 2001, the inhibitor titer was 0.4 BU. Treatment included immune tolerance therapy (dates and doses not provided) that was considered successful. On 28 February 2006, his FIX inhibitor antibody titer was 1.4 BU.

Subject G – Class II/III allergic reaction and inhibitor

Subject G was 5 years old at the time of chart review. This Caucasian male was diagnosed with severe haemophilia B on 05 May 1999 and had a transversion gene mutation, diagnosed when he was a newborn. The subject had an allergy to aspirin. The subject's family had no known allergies.

During a hospital admission for a central venous catheter placement, the subject began treatment with rFIX on 09 December 1999 continuing through 13 December 1999 (5 EDs). On 20 December 1999, the subject was started on prophylactic rFIX therapy.

Immediately following an infusion of rFIX, the subject experienced an anaphylactoid reaction. Within 5 minutes of the infusion, he developed an urticarial rash over his trunk and face. Treatment included IV diphenhydramine (twice) and IV hydrocortisone. Other medications administered during the event were IV ceftriaxone and vancomycin. The subject was transferred to the pediatric intensive care unit (PICU). He maintained good oxygen saturation with no evidence of bronchospasm. The reaction resolved within 24 hours. On 21 December 1999, the subject was rechallenged with 530 units of IV rFIX, and within 5 minutes of the infusion, he developed an erythematous rash.

Treatment included diphenhydramine, methylprednisolone, and ranitidine, which resulted in resolution of his rash. The subject recovered and was discharged from the hospital on 21 December 1999. The date of his last infusion prior to the reported event was 20 December 1999. No other events occurred. The subject was switched to rFVIIa (1.2 mg IV every 2 hours on demand), which he continued through 06 October 2003.

On 19 December 2000, the subject experienced an inhibitor, which was measured 1 year after the subject had been switched from rFIX to rFVIIa treatment and while the subject was on rFVIIa therapy (resolution date and titer information are unknown). The medical monitor assessed the event to be related to past rFIX treatment (December 1999). Because of multiple hospital admissions related to bleeding while the subject was receiving rFVIIa, it was decided that immune tolerance therapy (ITT) with rFIX should be initiated to better control his bleeding. On 29 September 2003, treatment with 2100 units of rFIX every other day began. On 16 October 2003, the subject developed hives at home after an rFIX infusion; the hives resolved without treatment. On 17 October 2003, the subject again developed hives at home after an rFIX infusion; he required 3 doses of diphenhydramine

for resolution. rFIX was not administered on 18 October 2003 or 19 October 2003. On 20 October 2003, the subject premedicated with diphenhydramine and came to the clinic for his rFIX infusion. He experienced a life-threatening anaphylactic reaction (as evidenced by hives, wheezing, respiratory distress and sinus tachycardia) and developed a cushingoid appearance. He was admitted to the PICU for monitoring.

The events were considered by the investigator and the medical monitor to be related to rFIX therapy. Treatment with IV diphenhydramine, hydrocortisone, ranitidine, and inhaled albuterol resolved the anaphylactic reaction the same day. ITT with rFIX continued in the hospital at an unknown dose until the subject was discharged on 16 November 2003. He continued with diphenhydramine given as premedication to rFIX for several months.

NOTE: The following events are past the study cutoff date and obtained from the subject's Adverse Experience Report: On 24 March 2004, the subject experienced inhibitor development of 1.4 BU. He continued ITT with rFIX at 3000 units daily until 03 September 2004. It was not known when ITT was discontinued, and the total number of EDs for the ITT was also unknown.

On 17 December 2004, the inhibitor resolved as the FIX antibody titer was <0.1 BU. The subject was desensitized and tolerized successfully. rFIX was continued on an alternate day basis, without any significant reactions.

Subject H - Class II/III allergic reaction

Subject H was 77 years old at the time of chart review. This male was diagnosed with moderate haemophilia B (date unknown). The subject's genotype and race were unknown.

The subject had a history of allergy to aminocaproic acid; no information was available regarding family history of allergic reactions. The subject had no history of inhibitor.

The subject received pdFIX (Mononine®) from 1992 to 1998 and rFIX from 08 July 1999 to November 2001 (EDs are unknown for each product). On 26 March 1996, he developed an allergic reaction during an infusion of pdFIX, which manifested with chest tightness, cyanosis, diaphoresis, dizziness, syncope and nausea. The investigator assessed these events to be of moderate severity, except the syncope, which was severe. All symptoms except the nausea were assessed as being related to pdFIX treatment.

The subject was rechallenged with pdFIX, and no additional allergic reactions occurred. He was treated with concomitant aminocaproic acid from March 1996 through 05 April 1996. The subject recovered. No other information is available regarding this event.

Subject I – Class II/III allergic reaction and inhibitor

Subject I was 12 years old at the time of chart review. This male of Caucasian and Pacific Islander origin was diagnosed with severe haemophilia B in 1993. The subject's genotype was identified as a total gene deletion. The subject had a history of allergies to bees and mosquitoes; his family allergy history was unknown. The subject received pdFIX (Bebulin® VH; Baxter International Inc.) from October 1993 to 17 January 1996 (16 EDs).

On 17 January 1996, the subject developed an allergic reaction during an infusion of Bebulin, which manifested with coughing, wheezing, facial edema, flushing and choking. He was treated with albuterol, diphenhydramine and oxygen, and the events resolved without sequelae. The investigator assessed all these events to be related to the pdFIX treatment; the severity of the events was not documented.

The subject was treated with Mononine® from 19 April 1996 to 15 August 1997 (19 EDs). On 15 August 1997, he developed an inhibitor (16 BU); the maximum titer (19.2 BU) was observed on 21 September 2001. No other information is available regarding this event. The subject was treated with FEIBA VH (15 EDs) from June 2001 to 27 November 2003. No other information on this subject is available.

Subject J – Inhibitor

Subject J was 13 years old at the time of chart review. This Caucasian male was diagnosed with severe haemophilia B in 1992. Information regarding the subject's genotype was unknown.

The subject had no history of allergies; his family history of allergies was unknown. He received pdFIX (AlphaNine®; Grifols Biologicals Inc.) from 03 February 1999 to 05 March 1999 (11 EDs). The subject began treatment with rFIX on 04 October 1999, and this treatment was noted to be ongoing at the time of this chart review (248 EDs). He also received pdFIX (Mononine®) from an unknown start date to 18 February 2000 (unknown EDs). No allergic reactions were documented while the subject was treated with AlphaNine, rFIX, or Mononine. The subject was reported to have developed an inhibitor; however, no information is available as to the date, etiology, treatment or resolution of the event.

Subject K – Inhibitor

Subject K was 11 years old at the time of chart review. This African American male had severe haemophilia B; the date of diagnosis is unknown. Information regarding the

subject's genotype, presence or absence of allergies and family allergy history were all unknown.

On 31 January 1996, prior to any recorded factor replacement therapy, the subject developed an inhibitor (1.4 BU). No other information was available regarding this event. The subject received pdFIX (AlphaNine®) from 24 February 1996 to 05 March 1997 (8 EDs). No allergic reactions were documented while he was treated with AlphaNine. The subject began treatment with rFIX on 15 August 1997, which was continuing at the time of this chart review (393 EDs). No allergic reactions were documented while the subject was treated with rFIX.