

## Recent trial results in recombinant coagulation factors for the treatment of hemophilia B

Hemophilia B is an inherited bleeding disorder, due to a deficiency in factor IX (FIX). Patients require an intravenously injected drug, known as clotting factor concentrate, to prevent or stop bleeding. In the past, patients relied on blood-based products and ultrapurified plasma-derived FIX, which carry the risk of infectious transmission. Technological advancements have led to the development of recombinant FIX (rFIX), which is free from that risk; however, patients still require frequent intravenous injections. Recent advancement involves the development of FIX concentrates with a longer half-life, resulting in the need for less frequent injections. This article will review the rFIX concentrates, BeneFIX® and Rixubis®, as well as the newer, longer acting products, rFIX-Fc (Alprolix™), N9-GP and rFIX-FP.

**Keywords:** Alprolix™ • BeneFIX® • Factor IX • hemophilia B • N9-GP • recombinant FIX • rFIX-FP • Rixubis®

Hemophilia B is an X-linked genetic disorder due to a mutation in the factor IX (FIX) gene which codes for FIX, an important coagulation protein. This mutation can lead to serious and potentially life-threatening bleeding due to a failure of secondary hemostasis. Patients with mild (>5% factor activity) or moderate (1–5% factor activity) hemophilia bleed most commonly after a traumatic injury or perioperatively. Patients with severe (<1% factor activity) hemophilia can spontaneously bleed into their joints, muscles, soft tissues, internal organs or into their CNS. The most common bleeding events occur in the joints, and primarily in the elbows, knees and ankles. Repeated bleeding episodes into joints, called hemarthrosis, will lead to permanent joint damage, a condition known as hemophilic arthropathy. In order to prevent hemophilic arthropathy as well as other, more rare, but life-threatening bleeds such as intracranial hemorrhages, patients must infuse factor replacement products intravenously on a regular basis – two- to three-times per week for hemophilia B.

Before the development of FIX concentrates, patients were given transfusions of whole blood or plasma for bleeding episodes [1]. These were later replaced by more effective prothrombin complex concentrates, a plasma-derived product with a high concentration of FIX [2]. In many parts of the developing world, plasma or less commonly, prothrombin complex concentrates remain the only form of replacement for FIX deficiency.

The 1970s marked the beginning of modern hemophilia treatment with the discovery of concentrated factor from pooled human plasma. The concentrated factor products could be stored at home, enabling ready and rapid access for patients to treat their bleeding episodes, resulting in improved hemophilia care [3]. This was also the time when prophylactic administration of factor became possible. Prophylaxis, as opposed to episodic treatment, has the potential to prevent hemophilic arthropathy and thus reduce morbidity and mortality and improve a patient's quality of life [4].

Unfortunately, in the 1980s, the large pools of human plasma from which these concen-

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trates are made were contaminated with the human immunodeficiency virus and the hepatitis C virus. The development of high-purity virus-inactivated plasma-derived FIX concentrates arose in the late 1980s as a result of this contamination, which claimed the lives of thousands of persons with hemophilia [3]. Despite these improvements in the safety of these products, the concern for product contamination with these and other infectious agents, such as prion diseases and non-enveloped viruses such as hepatitis A and parvovirus remained because they could not escape the viral inactivation technology [5]. As a result, and following the cloning of the FIX gene in 1982 [6], it became possible to synthesize FIX utilizing recombinant DNA technology to create products no longer subjected to the risks associated with human blood-based products. Currently, the recombinant factor IX (rFIX) concentrates, which were first introduced in 1998 [7], are the overwhelming choice for managing hemophilia by patients and physicians alike in countries that can afford the high cost of these products.

This paper will focus on trial results of two currently available standard rFIX products, as well as results from the most recent advancement of factor replacement, the so-called long-acting rFIX products. For patients who infuse rFIX prophylactically, the long-acting rFIX products can potentially decrease infusions from two- to three-times per week to once every 1–2 weeks. The standard products include recombinant factor IX (BeneFIX®, Pfizer, NY, USA) and Bax326 (Rixubis®, Baxter, IL, USA), and the longer acting products include rFIXFc (Alprolix™, Biogen Idec, MA, USA) and two products in development, rIX-FP (CSL Behring, PA, USA) and N9-GP (Novo Nordisk A/S, Bagsvaerd, Denmark).

Highlights from all product trials will be reviewed, including the standard rFIX products and how their development led to the newer, longer acting recombinant products (Table 1). The ongoing challenges of hemophilia care will also be discussed as well as the unmet needs of these patients.

### BeneFIX®

The cloning of the FIX gene in the 1982 enabled products like BeneFIX, to be created. BeneFIX is a recombinant FIX product that does not contain any human or animal sourced proteins during its manufacture or formulation. It is synthesized in Chinese hamster ovary (CHO) cells, which is a well-known mammalian cell line [21]. CHO cells are ideal for production of recombinant proteins because they can be grown in large-scale culture systems void of animal and human materials, and they have the capacity for post-translational modification. In order to provide additional viral inactivation, BeneFIX also undergoes a chromatography purification process and membrane nanofiltration, that has the ability to retain molecules with molecular weights >70,000 Da (large proteins and viral particles) [8,22].

### Phase I PK study

#### Study design & pharmacokinetic assessment

This Phase I trial evaluated the pharmacokinetics (PK) in 11 previously treated patients (PTPs) with severe or moderately severe hemophilia B [8]. The patients were randomized to receive 50 IU/kg of either BeneFIX or plasma-derived FIX (pdFIX) with a washout period in between. The mean recovery of rFIX was 0.84 IU/dl per IU/kg, which was 72% of the recovery of pdFIX (1.17 IU/dl per IU/kg) (Table 2). The statistically significant ( $p < 0.05$ ) difference in recovery was thought to be

Table 1. Summary of landmark clinical trials of recombinant factor IX products.

Product	Type of study	Number of patients	Ages (years)	Publication year	US FDA approved	Ref.
BeneFIX®	Phase I, double-masked, randomized, crossover, PK	11	Unknown	1997	Yes	[8]
BeneFIX	Phase I/II, PK and safety/efficacy	57	4–56	2001	Yes	[7]
BeneFIX	Phase II/III, open-label, single-cohort	63	0.08–14	2004	Yes	[9]
Rixubis®	Phase I/III, prospective, controlled	73	12–59	2014	Yes	[10–11]
Rixubis	Phase III, prospective, controlled	14	≥16	2014	Yes	[11–13]
Alprolix™	Phase I/IIa, open-label, dose escalation	14	21–64	2011	Yes	[14]
Alprolix	Phase III, nonrandomized, open-label	123	12–71	2013	Yes	[15]
N9-GP	Phase I, open-label dose escalation, PK	15	21–55	2011	No	[16]
N9-GP	Phase III, PK and blinded	74	13–70	2014	No	[17]
rFIX-FP	Phase I, dose escalation	25	15–58	2012/2013	No	[18,19]
rFIX-FP	Phase I/II open-label	17	13–46	2013	No	[20]

PK: Pharmacokinetics.

due to the post-translational modification of BeneFIX. The elimination half-life was similar between the two products (~18 h).

This paper also discussed the preliminary data for the Phase I/II study, which is discussed below.

### Phase I/II study

#### Study design & pharmacokinetic assessment

The Phase I/II study enrolled 57 PTPs aged 4–56 years with severe or moderate hemophilia B. These patients underwent PK assessment; assessment of thrombogenicity, and safety and efficacy evaluation during treatment of or prophylaxis of bleeding episodes, including surgical procedures [7]. In this study, patients continued their home regimen of factor treatment (either on-demand or prophylaxis) after an initial baseline PK study. Patients on a prophylactic regime administered an average dose of 40.3 IU/kg; range: 13–78 IU/kg. Patients using factor for on-demand treatment, administered a dose ranging from <20 to 100 IU/kg, although the majority administered between 20 and 50 IU/kg.

PK results were similar in both the Phase I [8] and Phase I/II studies [7]. In the Phase I/II study, a baseline PK assessment was performed after giving a single dose of BeneFIX at 50 IU/kg. Patients had a washout of 7 days from any previous factor. The mean FIX recovery after an infusion of 50 IU/kg of factor was 0.75 IU/dl per IU/kg (range: 0.34–1.38 IU/dl per IU/kg) (Table 2). The lowest recovery was seen in patients younger than 15 years of age ( $0.66 \pm 0.22$  IU/dl per IU/kg).

The mean elimination half-life was 19.3 h (range: 11.1–36.4 h).

#### Bleeding events

19 PTPs administered BeneFIX prophylactically two- to three-times a week. 16 patients had a total of 203 hemorrhages; 64 were related to injury and 139 were spontaneous. 85 (61%) of the spontaneous hemorrhages occurred 72 h after their BeneFIX infusion. 27 (19%) of the spontaneous hemorrhages were within 48 h of BeneFIX infusion. Three patients did not experience breakthrough bleeding. Ninety-three percent of the bleeding episodes for the prophylactic patients were rated as having an ‘excellent’ or ‘effective’ response to factor.

For the on-demand patients, there were a total of 1796 hemorrhages and a total of 2641 infusions were given. 59% of the bleeds were hemarthroses, 25% soft tissue/muscle bleeds, 16% multisite or other bleeding. A total of 80.9% of the episodes were controlled with one infusion of BeneFIX (range 1–123 doses). A total of 90.9% of hemorrhages had an ‘excellent’ or ‘good’ response to BeneFIX treatment.

#### Adverse events

No subjects had any serious adverse events (AEs) related to BeneFIX (Table 3). Four subjects had a minor allergic reaction, although the antifactor IgE antibody assay was negative. The symptoms did not reoccur after repeated dosing of BeneFIX. One subject developed a low titer inhibitor. No patients had any evidence of viral transmission, and no patients had a thrombotic event.

Table 2. Pharmacokinetic results from recombinant factor IX trials.

Product and dose	Recovery IU/dl per IU/kg (SD)	Half-life hours (SD)	Clearance ml/h/kg (SD)	Ref.
BeneFIX® 50 IU/kg	0.8	18.1 (5.1)	UNK	[8]
BeneFIX 50 IU/kg	0.7	19.3	6.3 <sup>†</sup>	[7]
BeneFIX 50 IU/kg	0.7 (0.3) <sup>‡</sup>	UNK	UNK	[9]
Rixubis® 75 IU/kg	0.9 (0.2)	26.7 (9.5)	6.4 (1.3)	[11]
Alprolix™ 25 IU/kg	0.8 <sup>§</sup>	53.5 <sup>§</sup>	3.6 <sup>§</sup>	[14]
Alprolix 50 IU/kg	0.9 (0.2)	57.6 (8.3)	3.4 (0.8)	[14]
Alprolix 100 IU/kg	1.0 (0.1)	56.5 (14.1)	3.2 (0.7)	[14]
N9-GP 25 IU/kg	1.4 (0.04)	82.9 (18.1)	0.8 (0.1)	[16]
N9-GP 50 IU/kg	1.4 (0.4)	96.2 (41.8)	0.7 (0.2)	[16]
N9-GP 100 IU/kg	1.3 (0.2)	110.4 (17.5)	0.6 (0.1)	[16]
rFIX-FP 25 IU/kg	1.6 (0.2)	104.7 (55.1)	0.7 (0.5)	[18,19]
rFIX-FP 50 IU/kg	1.4 (0.3)	91.6 (20.7)	0.7 (0.2)	[18,19]
rFIX-FP 75 IU/kg	1.1 (0.2)	98.8 (17.5)	0.9 (0.2)	[18,23]

<sup>†</sup>Value from the FIX-Fc (Alprolix) Phase III study.  
<sup>‡</sup>Patients aged 1 month to 12 years.  
<sup>§</sup>SD not calculated due to n = 1 for patients receiving 25 IU/kg of FIXFc (Alprolix).  
 PK: Pharmacokinetics; PUP: Previously untreated patient.

Table 3. Trial results of adverse events and bleeding episodes for each factor IX product.

Product	Type of study	AEs	SAEs	Product related SAEs	Inhibitor development	Allergic reaction	Severe allergic reaction	Thrombosis
BeneFIX®	Phase I/II	55	0	NA	1	4	0	0
BeneFIX	Phase II/III	22	4	4	2	8	2	0
Rixubis®	Phase I/III	90	0	NA	0	0	NA	0
Rixubis	Phase III	14	0	NA	0	0	NA	0
Alprolix™	Phase I/IIa	16	2	0	0	0	NA	0
Alprolix	Phase III	88	13	1	0	0	NA	0
N9-GP	Phase I	10	1	1	0	1	1	UNK
N9-GP	Phase III	215	7	0	0	0	0	0
rFIX-FP	Phase I	22	0	NA	0	0	NA	0
rFIX-FP	Phase I/II	40	0	NA	0	0	NA	0

AE: Adverse event; NA: Not applicable; SAE: Serious adverse event; UNK: Unknown values.

### Surgical procedures

27 had 27 procedures performed while on the study. Surgeons reported that 98% of the procedures had 'excellent' or 'good' hemostasis. Three of the procedures were performed with a continuous infusion of BeneFIX in doses ranging from 4.3 to 8.6 IU/kg/h.

### Phase II/III study

#### Previously untreated patients

#### Study design & pharmacokinetic assessment

This study evaluated the safety and efficacy of BeneFIX in previously untreated patients (PUPs), with moderate or severe hemophilia B without a history of inhibitors [9]. The study was performed on 63 patients aged 1 month to 14 years. PK studies were performed after a dose of 50 IU/kg of BeneFIX. Patients also received BeneFIX on-demand for bleeding episodes or prophylactically at a dose determined by the investigator.

The mean FIX recovery after receiving a mean dose of  $64.2 \pm 28.1$  IU/kg of BeneFIX (performed in 59 patients) was  $0.68 \pm 0.27$  IU/dl per IU/kg, which was consistent throughout children aged 1 month to 12 years (Table 2). Patients less than 1 month of age had a mean recovery of 0.46 IU/dl per IU/kg and those greater than 12 years of age had a mean recovery of  $0.93 \pm 0.41$  IU/dl per IU/kg. Unfortunately, there were not enough patients in these age groups to allow statistical comparison.

### Bleeding events

32 of the 42 patients who received BeneFIX for prophylaxis were on a routine prophylactic regime. 24 patients received BeneFIX two or more times a week and eight received BeneFIX once weekly. 27 (84%) patients had 246 bleeding episodes; 175 (76%) were injury-related

and 56 (24%) were spontaneous. 50 (89%) of the spontaneous hemorrhages occurred more than 48 h after infusion. Five (16%) patients did not have any breakthrough bleeding. 91% of the bleeding episodes for the prophylactic patients were rated as having an 'excellent' response to factor.

For the on-demand patients analyzed, there were a total of 997 hemorrhages and a total of 1505 infusions were given. 33% of the bleeds were hemarthroses, 49% soft tissue/muscle bleeds, 18% multisite or other bleeding. 75% of the episodes were controlled with one infusion of BeneFIX. 94% of hemorrhages had an 'excellent' or 'good' response to BeneFIX treatment. 1% of the bleeding episodes had 'no response' with the first BeneFIX infusion.

### Adverse events

17% of the patients had AEs that were deemed related to BeneFIX, most of which were mild or moderate (Table 3). Five patients in the study experienced allergic-type reactions, two of which (3%) developed FIX inhibitors with a titer of greater than 5 BU/dl. There were four serious AEs related to BeneFIX, two were the patients who developed inhibitors and the other two experienced dyspnea (one patient) and rigors (one patient). No patients had thrombotic events or transmission of a virus while on the study.

### Surgical procedures

BeneFIX was also tested in 23 PUPs during 30 surgical procedures, which included placement of 12 central venous access devices, seven circumcisions, one dental procedure and ten 'other' procedures. 97% of the procedures were reported as having 'excellent' or 'good' hemostasis.

**Reformulated study**

BeneFIX was reformulated to increase the ionic strength of the product by changing the diluent from sterile water to 0.234% NaCl [24]. The reformulation was done due to reports of agglutinated red blood cells in the intravenous tubing when the product was mixed with blood in the administration tubing and/or syringe. There were no reports of clinical sequelae with this agglutination.

**Study design & pharmacokinetic assessment**

This study evaluated the safety and efficacy of reformulated BeneFIX in 34 PTPs with moderate or severe hemophilia B without a history of inhibitors. Patients were aged 12–61 years (mean 28.3 years). The initial PK study was a double-blind, randomized, crossover study. Patients were randomized to receive a single 75 IU/kg dose of BeneFIX or reformulated BeneFIX. After a 5-day washout, they received the alternative BeneFIX product. After the PK study, patients received reformulated BeneFIX either as prophylaxis or on-demand as prescribed by the investigator. The on-demand dose ranged from 30.3 to 147.2 IU/kg, with a median dose of 87.4 IU/kg. PK studies were then repeated after patients had at least 30 exposure days.

The PK studies were very similar between the two products. The mean recovery for original BeneFIX was 0.68 IU/dl per IU/kg ( $\pm 0.18$ ) and recovery for reformulated BeneFIX was 0.73 IU/kg ( $\pm 0.2$  IU/kg). The half-life for original BeneFIX was 23.4 h ( $\pm 5.2$  h) and for reformulated BeneFIX it was 22.4 h ( $\pm 5.3$  h).

**Bleeding events**

17 patients received reformulated BeneFIX for routine prophylaxis during the open-label portion of the study. Six patients did not have any bleeding episodes, and 11 patients had 26 bleeds. 73% of the hemorrhages were due to injury, 23% were spontaneous and one was unknown. 73% of the bleeds were greater than 48 h after their reformulated BeneFIX infusion.

In total, there were 95 hemorrhages treated with 124 infusions for both the prophylaxis and on-demand therapy patients. 58% of the hemorrhages were due to injury, 41% were spontaneous and 1% were unknown bleeding events. 50% of the bleeding episodes were in a joint, 38% were in a soft tissue or muscle, 10% were multisite and 1% were hematuria. Over 80% of the hemorrhages were resolved with a single infusion and the infusion was rated as excellent or good for 85.3% of the hemorrhages.

**Adverse events**

The study did not report the total AEs for reformulated BeneFIX. There were two serious AEs, nei-

ther were product related. There were no reports of allergic manifestations, inhibitor development or thrombosis.

**Surgical procedures**

There was one surgical procedure during the study. This patient used 37 doses of reformulated BeneFIX over 2.9 weeks with an average dose of 24.6 IU/kg.

**Rixubis**

Rixubis (Bax326) is another rFIX product available on the market, which has a similar half-life to BeneFIX. Its recent approval in 2013 added increased treatment choices for rFIX replacement to the hemophilia community.

Rixubis was also developed using CHO cell clones grown in culture media free of human or animal products. Due to the susceptibility of CHO clones to small enveloped and nonenveloped viruses, Rixubis has two additional viral inactivation steps, a solvent/detergent treatment and a smaller sized nanofiltration of 15 nm [10,12].

**Phase I/III study****Study design & pharmacokinetic assessment**

The Phase I/III study was a prospective, controlled assessment of PK, efficacy, safety and immunogenicity in PTPs [10–11,13]. The 73 PTPs who received Rixubis in the trial were aged 12–59 years with moderate or severe hemophilia B without inhibitors who had previously been treated with pdFIX or BeneFIX. Subjects received Rixubis either as twice-weekly prophylaxis (median dose 50.5 IU/kg) or on demand.

A preliminary, randomized, blinded PK crossover study comparing Rixubis with BeneFIX was also performed. Subjects received an infusion of either Rixubis or BeneFIX at  $75 \pm 5$  IU/kg with a 5–7 day washout in between. The PK parameters between Rixubis and BeneFIX were comparable. The mean recovery was  $0.87 \pm 0.22$  IU/dl per IU/kg for Rixubis and  $0.76 \pm 0.20$  IU/dl per IU/kg for BeneFIX (Table 2). Their half-lives were also similar,  $26.7 \pm 9.55$  h for Rixubis and  $27.87 \pm 9.22$  h for BeneFIX. The mean clearance was found to be  $0.064 \pm 0.013$  dl/h/kg in Rixubis and  $0.068 \pm 0.015$  dl/h/kg in BeneFIX, which was also comparable.

**Bleeding events**

56 patients received Rixubis for prophylaxis twice weekly. There were 115 bleeding episodes in 32 patients. 24 (42.9%) patients on prophylaxis did not have any bleeding episodes during at least a 3-month period. Out of the patients who received on-demand treatment, there were 134 bleeding events in 14 subjects.



For all patients, 211 (84.7%) of bleeding events were controlled with one to two infusions. There were 130 spontaneous bleeding episodes, 90 injury-related and in 29 bleeds the cause was unknown. Hemostasis was rated as 'excellent' or 'good' in 96% of the episodes.

#### Adverse events

There were 90 AEs during the study, although only three, which were nonserious, were thought to be related to Rixubis (Table 3). There were no allergic reactions, thrombotic events or serious AEs related to the product. No patients developed an inhibitor or a treatment-related specific antibody.

#### Surgical procedures

Please see the following, Phase III study for surgical procedures.

#### Phase III study

##### Study design & pharmacokinetic assessment

This Phase III study was to evaluate the hemostatic efficacy and safety of Rixubis in 14 PTPs undergoing surgery, 13 of whom were enrolled in the previous Phase I/III parent study [11–13]. The study was designed in two segments, pre-operative and intra-/post-operative regimes. Pre-operatively, patients were given a loading dose of Rixubis to raise the FIX level to 80–100% of baseline for major procedures, and 30–60% of baseline for minor procedures. For major procedures, patients were given Rixubis to maintain their FIX trough level at 80–100% of baseline until wound healing was achieved. Once wound healing was achieved, FIX trough levels were maintained at 30–60% for 7–14 days.

#### Bleeding events

There were no reported bleeding events outside of intraoperative surgical bleeding.

#### Adverse events

No patients experienced a serious AE, had an allergic reaction or a thrombotic event (Table 3). No patients acquired an inhibitor or developed antibodies to Rixubis during the study. There were 14 nonserious AEs, which were unrelated to treatment with Rixubis.

#### Surgical procedures

Patients had major surgeries, including orthopedic surgeries, as well as minor surgeries and dental procedures. Hemostatic levels of FIX were achieved peri- and postoperatively in 100% of patients. Surgeons rated the hemostasis as 'excellent' or 'good.'

#### Pediatric study

A study evaluating the PK, efficacy, safety and immunogenicity of Rixubis in PTPs less than 12 years of age with severe or moderately severe hemophilia is currently ongoing [25].

#### Alprolix™

Alprolix™ (rFIXFc) is the first FDA-approved rFIX product with a prolonged half-life. Alprolix is made of a single molecule of FIX recombinantly attached to the human IgG Fc domain. The plasma half-life is prolonged by the interaction of the IgG Fc domain with the Fc receptor and the intracellular IgG recycling pathway which delays lysosomal degradation of the fusion protein, resulting in the molecule being recycled back into circulation [26].

#### Phase I/IIa study

##### Study design & pharmacokinetic assessment

The Phase I/IIa trial was an open-label, dose-escalation study of a single dose of Alprolix to evaluate the safety and PKs in PTPs with severe hemophilia B [14]. There were 14 patients who received Alprolix, all of whom were over 18 years of age and without a history of inhibitors or allergic reactions to FIX concentrates.

There were six sequential dose levels given to the patients, from 1 to 100 IU/kg (1, 5, 12.5, 25, 50 or 100 IU/kg). The PK data were taken from subjects given doses 25 (n = 1), 50 (n = 5) and 100 (n = 5) IU/kg. The recovery for doses 25-, 50- and 100 IU/kg were 0.77 IU/dl per IU/kg,  $0.87 \pm 0.21$  IU/dl per IU/kg and  $1.02 \pm 0.11$  IU/dl per IU/kg, respectively, with an average recovery of  $0.93 \pm 0.18$  IU/dl per IU/kg (Table 2). The half-life was 53.5 h,  $57.6 \pm 8.27$  h and  $56.5 \pm 14.1$  h for 25, 50 and 100 IU/kg respectively. Based on model prediction, the time for patients to reach a FIX level of 1% following an infusion of Alprolix took 7.34 days,  $10.1 \pm 1.58$  days and  $12.3 \pm 2.49$  days for doses 25, 50 and 100 IU/kg, respectively. For patients to reach a predicted FIX level of 3%, it took 3.81 days,  $6.28 \pm 1.11$  days and  $8.53 \pm 1.58$  days for doses 25-, 50- and 100 IU/kg. At 168 h (1 week) after the Alprolix was given, the average FIX activity was 1.11 IU/dl,  $2.47 \pm 0.91$  IU/dl and  $4.65 \pm 1.73$  IU/dl for the 25, 50 and 100 IU/kg dose groups, respectively.

#### Bleeding events

Six patients experienced bleeding episodes 9–28 days after factor dosing, when Alprolix had been washed out.

#### Adverse events

There were 16 AEs in this trial, most of which were mild or moderate (Table 3). The two treatment-related

AEs were dysgeusia and headache. There were two serious AEs comprising abdominal adhesions and depression, however neither were considered to be related to the factor product. No patients had allergic reactions, inhibitor formation or thrombosis.

### Surgical procedures

No surgical procedures were included in this study.

### Phase III study

#### Study design & pharmacokinetic assessment

The Phase III trial was a nonrandomized, open-label study evaluating the safety, efficacy and PKs of Alprolix for prophylaxis, treatment of bleeding episodes and perioperative management [15]. This study included 123 study subjects, aged 12 years of age or older, with severe hemophilia B (FIX  $\leq 2\%$ ) without a history of allergy to FIX or inhibitors.

The subjects were assigned to four treatment groups based on the clinical site's standard of care. Group 1 received a weekly dose of Alprolix at 50 IU/kg. Group 2 received 100 IU/kg of Alprolix every 10 days. The dose of factor for group 1 and the time interval in group 2 were adjusted as needed to maintain a trough FIX activity of 1–3 IU/dl, or higher. Group 3 received Alprolix on-demand for bleeding (20–100 IU/kg). Group 4 received Alprolix perioperatively.

A subgroup of 22 patients in group 1 underwent comparative, sequential pharmacokinetic assessments comparing BeneFIX to Alprolix. The PK data for Alprolix was comparable to the Phase I/II study, although the data comparing the efficacy to BeneFIX provided additional information. The mean recovery of Alprolix and BeneFIX was quite similar in this study, 0.92 IU/dl per IU/kg and 0.95 IU/dl per IU/kg, respectively. It took 5.8 days to reach a FIX level of 3% with Alprolix compared with 2.8 days with BeneFIX (Figure 1). The time to reach a FIX level of 1% was 11.2 days with Alprolix and 5.1 days with BeneFIX.

In group 2, 53.8% of the subjects were able to stretch their dosing intervals to 14 days or more during the last 3 months of therapy to maintain a trough of 1–3 IU/dl.

### Bleeding events

63 patients were in group 1 and received once weekly prophylaxis with Alprolix, and 23% did not have any bleeding episodes. 29 patients were in group 2 and received the interval-adjusted prophylaxis, and 42.3% did not have any bleeding episodes during the study.

When evaluating the data from the four groups together, there were 636 bleeding episodes, and 90.4% of patients required only 1 injection of Alprolix to resolve the bleed. The median dose per injection was

46 IU/kg and the median time between injections, if a second dose was given, was 45 h. The study did not comment on the description of the bleeding episodes.

### Adverse events

74% of the patients had at least one AE in groups 1, 2 and 3 and 10.9% were reported as serious AEs (Table 3). Most of these events were consistent with those expected in patients with hemophilia. One serious AE that was considered possibly related to Alprolix was an obstructive ureteral clot causing painful hematuria.

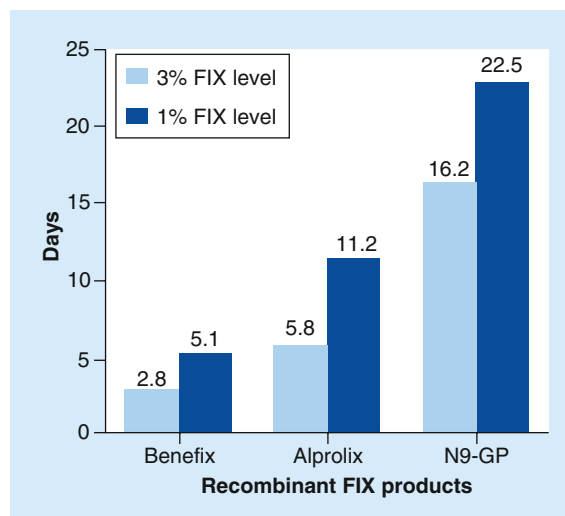
No inhibitors were detected during this study. Non-neutralizing antibodies were detected at a low titer level in three patients prior to receiving the study drug, which all became negative during the study. One patient had a borderline negative antibody prior to the study and had a borderline positive non-neutralizing antibody at the end of study. None of the antibodies affected the PKs nor did they have any apparent clinical effects. There were no reports of vascular thrombosis, serious hypersensitivity events or anaphylaxis.

### Surgical procedures

There were 14 major surgeries in 12 patients during the study. Hemostasis was rated as excellent or good during all major surgeries.

### N9-GP

N9-GP is a glycoPEGylated rFIX product that is currently in the late stages of drug development. This prod-



**Figure 1. Time to reach mean factor IX activity of 3 and 1% above their baseline after an infusion of 50 IU/kg of either BeneFIX®, Alprolix™ or N9-GP.** Data for BeneFIX and Alprolix are taken from the Phase III Alprolix trial. Data for N9-GP are taken from its Phase I pharmacokinetic trial. FIX: Factor IX.

uct is also made in CHO cells in order to synthesize the rFIX part of N9-GP, and its amino acid sequence is identical to BeneFIX. A 40-kDa polyethylene glycol (PEG) molecule is attached to the rFIX activation peptide to make N9-GP such that when the molecule is activated, the PEG is cleaved off, thus leaving the activated FIX [16]. There are approximately 20 currently approved medications that use PEGylation technology to increase the circulation time of certain drugs by interfering with renal clearance and glomerular filtration [27].

### Phase I PK study

#### Study design & pharmacokinetic assessment

This open-label, dose-escalation trial was the first human trial evaluating the safety and pharmacokinetic properties of three ascending doses of N9-GP [16]. 16 PTPs, ranging in age from 21 to 55 years, with hemophilia B ( $\leq 2\%$  FIX activity), without a history of inhibitors were enrolled in this study. 15 patients completed the trial. Patients first received one dose of 25, 50 or 100 IU/kg of their normal factor concentrate (either pdFIX or BeneFIX). They then underwent a washout period of 10 days followed by an infusion of the same dose/kg of N9-GP.

The mean incremental recovery of N9-GP was 1.33 IU/ml per IU/kg (Table 2), which was significantly higher compared with BeneFIX (0.68 U/ml per U/kg). The recovery for the 25, 50 and 100 IU/kg dosing of N9-GP was  $1.4 \pm 0.04$  U/ml per U/kg,  $1.39 \pm 0.44$  U/ml per U/kg and  $1.28 \pm 0.23$  U/ml per U/kg, respectively. The mean half-life of N9-GP was 93 h, which was approximately five-times longer than BeneFIX (19 h). The half-life for the 25, 50 and 100 IU/kg dosing of N9-GP was  $82.94 \pm 18.15$  h,  $96.25 \pm 41.85$  h and  $110.45 \pm 17.48$  h, respectively. The mean clearance of N9-GP was 0.70 ml/h/kg, which was about tenfold slower than BeneFIX.

The estimated time to 1% FIX activity above the baseline in patients who received 50 IU/kg of N9-GP was 22.5 days, and the time to 3% above baseline was 16.2 days (Figure 1). These estimated results should be interpreted with caution because after 168 h, FIX activity was only measured at 2 and 4 weeks after their dose.

### Bleeding events

No discussion of bleeding events was included in this study.

### Adverse events

There were 11 adverse events of which ten were moderate or mild (Table 3). The one severe event was an allergic reaction during the drug administration and was most likely related to N9-GP. No patients on the trial developed inhibitors.

### Phase III study

#### Study design & pharmacokinetic assessment

This randomized, single-blind trial investigated the pharmacokinetics, safety and efficacy of nonacog  $\beta$  pegol (N9-GP) [17]. Seventy-four PTPs with hemophilia B ( $\leq 2\%$  FIX activity) aged 13–70 years old and no history of inhibitors were enrolled on the Phase III. There were two arms to the study, the prophylaxis arm and the on-demand arm. Patients on prophylaxis were randomized 1:1 in a blinded fashion to receive once weekly dosing of either 10 IU/kg or 40 IU/kg of N9-GP for 52 weeks. On-demand patients were treated for 6 months, and were given 40 IU/kg of N9-GP for mild/moderate bleeding events and 80 IU/kg for severe bleeding. If a patient on prophylaxis had a bleeding episode, he also used the same dosing regime for mild/moderate and severe bleeding.

The single dose half-life was 93 h (CV% 19.5) and the steady state half-life was 107 h (CV% 21.8) in the 10 IU/kg arm. For the 40 IU/kg arm, the single dose half-life was 85 h (CV% 21.8) and the steady-state half-life was 111 h (CV% 11.8).

### Bleeding events

30 patients administered 10 IU/kg N9-GP once weekly and there were 132 bleeding episodes in 25 patients. 91 (68.9%) of the bleeding episodes were spontaneous, 39 (29.5%) were traumatic, 1 (0.8%) were after minor surgery and 1 (0.8%) was other. 111 (84.1%) of the hemorrhages resolved with one injection of N9-GP.

29 patients administered 40 IU/kg N9-GP once weekly and there were 70 bleeding episodes in 16 patients. 34 (48.6%) of the bleeding episodes were spontaneous and 36 (51.4%) were traumatic. 69 (98.6%) of the hemorrhages resolved with one injection of N9-GP.

15 patients administered N9-GP on-demand and there were 143 bleeding episodes in 14 patients. 102 (71.3%) of the bleeding episodes were spontaneous and 41 (28.7%) were traumatic. 120 (83.9%) of the hemorrhages resolved with one injection of N9-GP.

78% of the bleeding episodes were in joints, and 92.2% had a 'successful' treatment of their bleed. For those patients receiving 40 IU/kg once weekly for prophylaxis, 99% of their bleeding episodes were treated with one infusion.

### Adverse events

Overall, the study found N9-GP to be well tolerated and safe. No patients in the study developed an inhibitor, had a thrombotic event or an allergic reaction related to N9-GP (Table 3). There were 215 AEs and seven severe events. The serious events were unlikely related to N9-GP.



**Surgical procedures**

No surgical procedures were included in this study.

**rFIX-FP**

rFIX-FP is also produced using CHO cells to make the rFIX portion, with a recombinant albumin genetically fused to the C-terminus of rFIX. A linker sequence is placed between the rFIX and albumin portion, which contains the same cleavage site that activates FIX. Thus, when FIX is activated, the linker sequence is cleaved and the albumin portion is released [23]. The creators of rFIX-FP hypothesize that this product should not cause activation of the immune system since albumin is a natural plasma protein and carrier molecule [18].

**Phase I dose-escalation study****Study design & pharmacokinetic assessment**

The first, in human dose-escalation study assessed the safety and PKs of rFIX-FP in 25 patients with hemophilia B ( $\leq 2\%$  FIX activity), aged 15–58 years, without a history of inhibitors [18,19]. One subject in the study was younger than 18 years. Patients were divided into groups to receive either 25, 50 or 75 IU/kg of rFIX-FP. 15 of the subjects in the 50 IU/kg also received a single dose of 50 IU/kg of their previous FIX product, either pdFIX or rFIX (BeneFIX) after a 4-day washout period.

The incremental recovery for rFIX-FP at doses 25-, 50- and 75 IU/kg was  $1.65 \pm 0.19$  IU/dl per IU/kg,  $1.38 \pm 0.28$  IU/dl per IU/kg and  $1.08 \pm 0.19$  IU/dl per IU/kg, respectively (Table 2). The mean incremental recovery of rFIX-FP was 29% higher than the recovery of 50 IU/kg of BeneFIX at  $0.94 \pm 0.24$  IU/dl per IU/kg. The half-life of each rFIX-FP dosing was  $104.71 \pm 55.08$  h,  $91.57 \pm 20.74$  h and  $98.82 \pm 17.48$  h for dosing groups 25-, 50- and 75 IU/kg, respectively. In this study, the half-life for BeneFIX was found to be similar to other studies at  $17.23 \pm 2.28$  h. The FIX activity level at 14 days after patients were given a dose of 25-, 50- and 75 IU/kg of rFIX-FP was  $2.5\% \pm 2.66$ ,  $5.54\% \pm 2$  and  $6.01\% \pm 2.45$ , respectively. Similar FIX activity levels were seen at 48 h after patients were given 50 IU/kg of BeneFIX ( $5.96 \pm 1.07\%$ ).

The clearance, as expected for rFIX-FP, was much slower when compared with the clearance of BeneFIX at  $5.24 \pm 0.85$  ml/h/kg. For the 25-, 50- and 75 IU/kg dosing, the clearance was  $0.73 \pm 0.46$  ml/h/kg,  $0.75 \pm 0.19$  ml/h/kg and  $0.87 \pm 0.17$  ml/h/kg, respectively.

**Bleeding events**

There were 18 bleeding episodes in 12 subjects during the study. There were nine spontaneous hemorrhages

in six patients. Two of these episodes occurred on days 14 and 15 in the 25 IU/kg group when the patient's FIX activity level returned to baseline, and seven of the episodes occurred during the screening period or during the safety follow-up period after the administration of the previous FIX product during the PK assessment. None of the episodes occurred during the 14-day PK study in the 50 and 75 IU/kg groups.

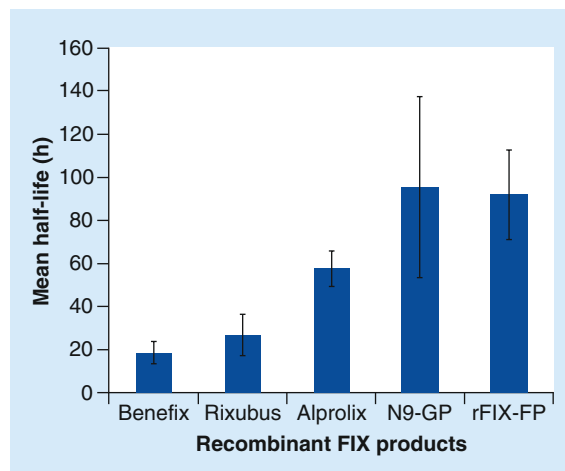
**Adverse events**

During this study, no patients developed inhibitors, had a hypersensitivity reaction or developed a thrombosis (Table 3). There were 22 reported AEs of which 21 of were mild. The moderate event was abdominal pain and possibly related to rFIX-FP.

**Phase I/II study****Study design & pharmacokinetic assessment**

The Phase I/II study evaluated the safety of rFIX-FP when given on-demand or prophylactically to 17 PTPs with hemophilia B ( $\leq 2\%$  FIX activity) aged 13–46 without inhibitors [20]. Five of the patients were children, aged 13–18 years.

Patients were given rFIX-FP either on-demand or as prophylaxis. To determine the prophylaxis schedule, patients were entered into a 10–14-day PK evaluation. Patients first received a dose of 25 IU/kg of rFIX-FP, and if on day 10 their FIX level was greater than 5%, then they received a second dose of rFIX-FP. Those whose FIX level was greater than 5% did not receive another dose of factor until day 14. The prophylaxis group initially received 25–35 IU/kg of rFIX-FP



**Figure 2. Comparison of the mean half-life for each recombinant factor IX product after patients received 50 IU/kg of factor.** Error bars represent standard deviation. Data were taken from Phase I pharmacokinetic trials for each individual factor product. Rixubis was not included due to half-life data based only on 75 IU/kg dosing. FIX: Factor IX.

weekly, but this gradually increased to 50–75 IU/kg weekly based on patient requirements up to 44 weeks. The on-demand group received 25 IU/kg of rFIX-FP as needed for bleeding.

The only PK data currently available for this study are the FIX activity was 4.4% on day 7 after patients received 25 IU/kg of rFIX-FP.

### Bleeding events

Efficacy for rFIX-FP is currently being analyzed. Preliminary results show that all bleeding episodes were treated successfully with rFIX-FP in the on-demand group.

### Adverse events

40 AEs occurred, but none were considered related to rFIX-FP (Table 3). No serious AEs were reported,

and there were also no hypersensitivity reactions or inhibitors that developed.

### Surgical procedures

No surgical procedures were included in this study.

### Future perspective

The treatment for hemophilia B has undergone significant and positive changes over the past few decades, first from human plasma derived factor to recombinant factor, and most recently to longer acting recombinant factor products. The recombinant products improved the safety of available FIX treatment, by removing the risk of viral or prion diseases, and the newer products are improving the quality of life of patients with hemophilia B by increasing the half-life (Figure 2).

## Executive summary

### BeneFIX® trials

- BeneFIX was the first nonhuman, virally inactivated, recombinant FIX product available as factor replacement for patients with hemophilia B.
- BeneFIX has an average half-life of 18–19 h.
- BeneFIX has a decreased recovery by about 30% in previously treated patients (PTPs) when compared with pdFIX, and the recovery is even lower in those patients less than 15 years of age.
- Clinical trials show it is safe and clinically effective in the treatment and prevention of bleeding episodes in PTPs and previously untreated patients with hemophilia B.
- Trials did not show an increased risk of inhibitor development in PTPs, but in the previously untreated patient trial, the incidence of inhibitor development was 3%.

### Rixubis® trials

- Rixubis is the second rFIX product available for patients with hemophilia B. Its production involves two additional viral inactivation steps as compared with BeneFIX.
- Rixubis has a similar PK profile to BeneFIX, and the half-life is about 27 h.
- Trials showed an excellent safety profile, without severe allergic reactions, thrombotic events or development of inhibitors in PTPs. It was also shown to be clinically effective during bleeding episodes and perioperative management.

### Alprolix™ trials

- Alprolix™ (rFIXFc) is the first US FDA-approved rFIX product with a prolonged half-life. It is made by covalently fusing a single molecule of rFIX to the Fc domain of a human IgG molecule.
- Alprolix trials showed it is a safe and effective alternative for FIX replacement therapy, without inhibitor formation, allergic reactions or thrombogenicity in PTPs.
- Alprolix was found to have a prolonged mean half-life of over 50 h, compared with BeneFIX (~18 h), thus allowing dosing every 1–2 weeks to achieve effective prophylaxis.

### N9-GP trials

- N9-GP is another rFIX product with a prolonged half-life by adding a 40-kDa polyethylene glycol (PEG) molecule to the rFIX activation peptide.
- N9-GP has a half-life of up to 110 h, thus allowing prophylactic dosing of once weekly, or even less frequently.
- Trial results showed it was generally well tolerated, although one patient, out of 16, had an allergic reaction related to the product.
- Ninety nine percent of the bleeding episodes were treated with one dose of N9-GP.

### rFIX-FP trials

- rFIX-FP is also a rFIX product with a prolonged half-life. It is produced by fusing a recombinant human albumin to the C-terminus of rFIX.
- rFIX-FP has a mean half-life of 92 h and data suggest that once weekly infusion may be appropriate for prophylaxis, and some patients up to every 10–14 days.
- Trial results show an excellent safety profile, without any thrombotic or hypersensitivity events and no development of inhibitors.

Recent trial results from the longer acting rFIX products have shown that patients can maintain a FIX activity level of 1–3% with a range of weekly to biweekly infusions. There is hope (though as of yet no data) that with less frequent dosing, patients will be more compliant with their treatment, thus leading to decreased bleeding episodes and hemophilic arthropathy [28,29]. Furthermore, with less frequent dosing, patients (particularly young children) on prophylaxis may not require central venous catheters which could further improve quality of life, and reduce or even eliminate the morbidity and mortality associated with complications from central lines such as infections.

Thus far, the new products have demonstrated an outstanding safety profile, with only one incidence of an allergic reaction (to N9-GP), and no development of inhibitors in any of the long-acting products

in PTPs. The next step will be the evaluation of the safety, particularly as it relates to inhibitor formation in PUPs. Importantly, if the PK studies in PUPs show comparable results to PTPs, then there may indeed be a reduced reliance on central venous catheters in children with hemophilia B. These trials are currently under way.

#### Financial & competing interests disclosure

G Young has received honoraria and consulting fees from Novo Nordisk and Biogen Idec. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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