BAX326 (RIXUBIS): a novel recombinant factor IX for the control and prevention of bleeding episodes in adults and children with hemophilia B

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Abstract: Hemophilia B management has improved considerably since the introduction of high-purity plasma-derived factor IX (pdFIX) products in the early 1990s. Recombinant FIX (rFIX) was introduced more recently and has potential safety advantages over the older blood-based products. Until recently, only one such product, nonacog alfa (BeneFIX®, Pfizer, Inc.), has been available. However, a new rFIX product, BAX326 (RIXUBIS, Baxter Healthcare Corp.), has now been approved by the US Food and Drug Administration. BAX326 undergoes rigorous virus elimination and purification steps during manufacture, and has low activated FIX activity, which confers low thrombogenic potential in humans. Preclinical studies showed promising pharmacokinetic and safety profiles, and these early findings have since been expanded in a series of prospective, multicenter, clinical studies. Foremost among these is a pivotal phase I/III study of BAX326 and its use in routine prophylaxis or on-demand treatment in patients aged 12–65 years with severe (FIX level <1%) or moderately severe (FIX level ≤2%) hemophilia B. This study confirmed the pharmacokinetic equivalence of BAX326 and nonacog alfa, and showed a significant reduction in annualized bleeding rate with BAX326 prophylaxis compared with on-demand treatment (79% *versus* historic controls; $p < 0.001$). The hemostatic efficacy of BAX326 was rated as ‘excellent’ or ‘good’ in 96% of bleeds. BAX326 was also associated with statistically significant and clinically meaningful improvements in physical health-related quality of life. Results are similarly encouraging in a pediatric study in children aged up to 12 years and in a study in hemophilia B patients undergoing surgery. A further study showed safe transition, with no inhibitor formation in any patient, from treatment with a pdFIX product to BAX326. Overall, the safety profile of BAX326 in clinical trials has been strong, with no inhibitor or specific antibody formation, thrombosis, or treatment-related serious adverse events or anaphylaxis.

Keywords: health-related quality of life, hemophilia B, pediatric, pharmacokinetics, prophylaxis, recombinant factor IX, safety, surgery

Introduction

Hemophilia B is an X-linked hereditary bleeding disorder caused by congenital deficiency of coagulation factor IX (FIX) [Srivastava *et al.* 2013] (Figure 1, Mann, 1999). Several mutations have been described in the FIX gene, the most common of which include single base-pair changes associated with mis-sense, frameshift or nonsense mutations [Franchini *et al.* 2013]. Hemophilia B is less common than hemophilia A, which is linked

to factor VIII (FVIII) deficiency; of 172,373 people identified with hemophilia across 109 countries in the World Federation of Hemophilia (WFH) Global Survey of 2012, 28,008 (16.2%) had hemophilia B [WFH, 2013].

The severity of bleeding in hemophilia is related to the degree of clotting factor deficiency [Srivastava *et al.* 2013]. Data from the USA shows that approximately 36% of patients with

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Figure 1. Factor IX (FIX) and the classic coagulation cascade. FIX is activated (FIXa) proteolytically by factor XIa or factor VIIa to form factor IXa. Together with other cofactors, including factor VIIIa in addition to phospholipid and calcium ions, FIXa forms the tenase complex which activates factor X to form factor Xa [Mann, 1999].

hemophilia B have the severe form of the disease (FIX level <1% of normal; data for other countries may vary) [CDC, 2011], which is associated with spontaneous bleeding into joints and muscles, usually in the absence of any obvious trauma [Srivastava *et al.* 2013]. Repeated bleeding into joints without adequate treatment can result in crippling chronic joint disease, pain and reduced quality of life [Elander and Barry, 2003; Miners *et al.* 1999; Molho *et al.* 2000; Aznar *et al.* 2000]. In addition, some bleeding episodes, such as gastrointestinal and intracranial bleeds, can be life-threatening [Srivastava *et al.* 2013].

Although hemophilia A and B are considered to be clinically very similar [Makris, 2009], differences in bleeding phenotype have been suggested in severe cases [Makris, 2009], and the pharmacokinetics of FVIII and FIX differ from each other. Plasma-derived FIX (pdFIX) has a higher average clearance (approximately 4 *versus* 3 ml/h/kg) and longer elimination half-life (possibly in excess of 30 hours *versus* approximately 14 hours) than plasma-derived FVIII (pdFVIII) [Björkman and Berntorp, 2001]. Also, the volume of distribution at steady state (V_{SS}) of pdFVIII approximates to that of plasma, whereas the V_{SS} of pdFIX is reportedly 3–4 times plasma volume [Björkman and Berntorp, 2001].

Treatment of hemophilia B is underpinned by replacement of the deficient FIX as required for

bleeding episodes (on-demand treatment) or by scheduled dosing in order to maintain appropriate FIX levels and to reduce the risk of bleeding (prophylaxis) [Srivastava *et al.* 2013; Franchini *et al.* 2013]. In general, treatment of acute bleeding episodes with factor replacement for hemophilia B aims to achieve a plasma FIX level of 60–80% for major bleeds and 20–40% for minor bleeds [Franchini *et al.* 2013]. Reduction of bleeding episodes, with as close to zero bleeds as possible, is important in enhancing joint health and maximizing health-related quality of life (HRQoL) [Gringeri *et al.* 2014]. Accordingly, WFH guidelines recommend prophylaxis to prevent bleeding and joint destruction, and to preserve normal musculoskeletal function, and state that prophylaxis is advisable before patients take part in activities involving an increased risk of trauma [Srivastava *et al.* 2013]. Despite this, results from the 2006 Canadian National Haemophilia Prophylaxis Survey showed a considerably lower proportion of patients with hemophilia B than with hemophilia A were receiving prophylaxis (32% *versus* 69% of those with severe disease) [Biss *et al.* 2008]. This may be related, in part, to a perception that hemophilia B has a less severe bleeding phenotype and that fewer prophylaxis studies have been conducted in hemophilia B compared with hemophilia A [Biss *et al.* 2008; Schulman *et al.* 2008].

Primary prophylaxis (i.e. regular continuous prophylactic treatment, started before the second large

joint bleed and 3 years of age [Srivastava *et al.* 2013]) is associated with significant reductions in frequency of bleeding and joint damage *versus* episodic therapy in hemophilia A [Manco-Johnson *et al.* 2007]. Although some surrogate data on the need for orthopedic surgery in the longer term have been published [Mannucci and Franchini, 2013], equivalent joint outcomes information for patients with hemophilia B remains unavailable. There is currently no concrete evidence to suggest that patients with severe hemophilia B should not receive primary prophylaxis from the time of diagnosis or first episode of joint bleeding [Mannucci and Franchini, 2013]. Secondary prophylaxis refers to regular treatment started after ≥ 2 bleeds into large joints, but before the onset of joint disease, while tertiary prophylaxis refers to regular treatment started after the onset of joint disease to prevent further damage [Srivastava *et al.* 2013]. Although prophylaxis is often expensive, it is considered worthwhile because of the avoidance of future high costs associated with subsequent management of joint damage and the improvement of quality of life for patients [Srivastava *et al.* 2013].

Highly purified pdFIX concentrates were first introduced in the early 1990s when they represented an advance in hemophilia management over previously used prothrombin complex concentrates (PCC) and fresh frozen plasma (FFP) [Kim *et al.* 1992; Furie *et al.* 1994]. A large number of these agents have since become commercially available [Brooker, 2012] and the safety of these products has increased since their introduction due to improvements in sourcing and purification procedures for blood derivatives, and the introduction of multiple viral inactivation steps [Kim *et al.* 2008]. However, concerns remain over potential contamination by prions, unencapsulated viruses and other unknown pathogens [Ludlam and Turner, 2006; Key and Negrier, 2007; Dietrich *et al.* 2013]. More recently, recombinant FIX (rFIX) products, which have a lower risk of pathogenic contamination, have been developed [Franchini *et al.* 2013]. Until 2013, nonacog alfa (BeneFIX®, Pfizer Inc., New York, USA) [Pfizer Inc., 2014] was the only commercially available rFIX, but BAX326 (nonacog gamma, RIXUBIS; Baxter Healthcare Corp., Westlake Village, CA, USA) has now also been approved by the US Food and Drug Administration (FDA) and the Australian Therapeutic Goods Administration for use in adults [Baxter Healthcare Corp., 2014; Therapeutic Goods Administration, 2014]. Research is also ongoing into the use of

recombinant DNA technology and bioengineering to modify the pharmacokinetics of rFIX. Strategies include polymer modification with polyethylene glycol (PEG) and protein fusion technology [Østergaard *et al.* 2011; Negrier *et al.* 2011; Shapiro *et al.* 2012; Santagostino *et al.* 2012]. FIX replacement therapy in the USA primarily consists mostly of rFIX [WFH, 2013]. In Europe, however, approximately 50% of FIX usage remains plasma-derived, with broad variations in other geographies [WFH, 2013]. Furthermore, the proportion of patients with severe hemophilia B who receive prophylaxis is less than the corresponding proportion of severe hemophilia A patients, with fewer than half of severe hemophilia B patients receiving prophylaxis in the USA and Canada [CDC, 2011; Biss *et al.* 2008].

There is consequently a need to increase the worldwide availability of rFIX to support likely increases in demand, to ensure consistent supply and to increase treatment options. The introduction of BAX326 and other pipeline rFIX products represents a step towards realizing these goals. Moreover, BAX326 is the only FIX product currently approved in the USA for routine prophylaxis [Baxter Healthcare Corp., 2014; Pfizer Corp, 2014].

BAX326

The manufacture and preclinical activity of BAX326 have been covered in detail elsewhere [Dietrich *et al.* 2013; Baxter Healthcare Corp., 2014]; a brief summary follows.

BAX326 is developed using a recombinant Chinese hamster ovary (CHO) cell clone in suspension culture. Its amino acid sequence is identical to that of the Ala-148 allelic form of pdFIX, and its structural and functional characteristics are similar. No human or animal proteins are added at any stage of the manufacturing and formulation process. The CHO cell line secretes rFIX into a defined cell culture medium that does not contain hormones and the rFIX is purified by a chromatography purification process that does not require a monoclonal antibody step. Virus inactivation and removal is by solvent/detergent treatment and 15 nm nanofiltration, in contrast to nonacog alfa where nanofiltration alone is used. Potency of BAX326 is determined using an *in vitro* activated partial thromboplastin time (aPTT) based one-stage clotting assay calibrated against the World Health Organization (WHO) International Standard for FIX concentrate.

Virus elimination steps (including nanofiltration and solvent/detergent treatment) are included in the manufacture of BAX326 to reduce or eliminate the presence of small non-enveloped or enveloped viruses and any unknown pathogens [Dietrich *et al.* 2013; Berting *et al.* 2010; Rezvan *et al.* 2006]. Purification also removes recombinant furin, a proteolytic enzyme which is coexpressed with rFIX in CHO cells, and completes the required proteolytic cleavage of rFIX in order for it to be functional [Dietrich *et al.* 2013]. Preclinical studies have shown the hemostatic efficacy of BAX326 to be dose-related and consistent across batches, with rFIX activity declining over time in a biphasic manner (similarly to nonacog alfa) [Dietrich *et al.* 2013]. Results obtained in animals showed no signs of thrombogenesis or adverse clinical, respiratory or cardiovascular effects, and immunogenicity was similar to nonacog alfa [Dietrich *et al.* 2013].

The use of FIX products has been associated with the development of thromboembolic complications and the levels of activated FIX (FIXa) in FIX products demonstrated a high correlation with thrombogenicity in a rabbit stasis model [Gray *et al.* 1995]. The FIXa content in BAX326 (0.009 IU/mL) and nonacog alfa (0.106 IU/mL) was measured *in vitro* by a FIXa chromogenic assay [Turecek *et al.* 2013]. A separate study demonstrated with a chromogenic assay that rFIX (nonacog alfa) contained approximately 10-fold higher FIXa levels than pdFIX (AlphaNine): 12 ± 4 pM FIXa in pdFIX and 117 ± 22 pM FIXa in rFIX [Yu and Millar, 2014].

The thrombogenic potentials of BAX326 and nonacog alfa were evaluated in a Wessler rabbit stasis model, where a single IV injection of BAX326 or nonacog alfa was administered to six animals per treatment group. No thrombogenicity was observed with BAX326, as all animals in this treatment group received an assessment score of 0 on a 4-point thrombus formation scale [Dietrich *et al.* 2013]. When additional FIXa was spiked into BAX326, the mean Wessler score increased to 0.42, which confirms the correlation of Wessler score with FIXa content. The mean Wessler score of the nonacog alfa treatment group was 0.5, demonstrating the lower FIXa content of BAX326. Therefore, the risk of a thromboembolic event with the use of BAX326 in humans is considered to be similarly low or even lower than for the previously licensed rFIX.

Clinical development

The clinical development of BAX326 is currently based on five prospective, multicenter studies (Table 1). Briefly, the trials are:

- a pivotal phase I/III study of the pharmacokinetics of BAX326 and its use in routine prophylaxis or on-demand treatment in patients aged 12–65 years
- a phase II/III pediatric study of routine prophylaxis in children aged <12 years
- a continuation study of routine prophylaxis or on-demand treatment in patients aged up to 65 years
- a phase III study in patients aged 12–65 years undergoing surgical or other invasive procedures
- a phase IV study in patients aged <65 years where patients received the pdFIX product Immunine® (Baxter Healthcare Corp., Westlake Village, CA, USA) prior to entering the pivotal or pediatric studies in order to prospectively monitor the switch from a pdFIX to rFIX.

The clinical study program focuses not only on the hemostatic efficacy and safety of BAX326, but also on possible FIX inhibitor development when receiving or transitioning to BAX326. The development of inhibitors (alloantibodies to FIX) is the most challenging complication of FIX replacement therapy [Franchini *et al.* 2013], and affects between 1.5% and 3% of hemophilia B patients [DiMichele, 2007]. Inhibitors render FIX replacement therapy ineffective [Giordano *et al.* 2013] and place the patient at increased risk of allergic reaction after further exposure to FIX products [Srivastava *et al.* 2013].

Pivotal phase I/III study

The aims of this prospective study (250901) [ClinicalTrials.gov identifier: NCT01174446], which has recently been published [Windyga *et al.* 2014a, 2014c] were to assess the pharmacokinetic (PK) characteristics of BAX326 in comparison with nonacog alfa and to investigate the hemostatic efficacy, safety and HRQoL of BAX326 in previously treated patients aged 12–65 years with severe (FIX level <1%) or moderately severe (FIX level $\leq 2\%$) hemophilia B. The study was conducted in three parts: a double-blind, randomized, crossover, single dose PK investigation, followed by an open-label assessment of prophylaxis or on-demand

Table 1. Clinical study program for BAX326.

Study number (phase) and ClinicalTrials. gov identifier	Study	Patients	Design and dosing	Objectives	Reference(s)
250901 (phase I/III) NCT01174446	Pivotal study (pharmacokinetics, efficacy, and safety)	Age 12–65 years Severe/moderately severe disease Previously treated Immunocompetent No history of FIX inhibitors	Part 1: Pharmacokinetics R, DB, XO BAX326 versus BFX: 75 ± 5 IU/kg single dose; WO 5–7 days Part 2: Treatment and prophylaxis OL, BAX326 only Prophylaxis: 50 IU/kg (40–60 IU/kg; maximum 75 IU/ kg) twice weekly × 6 months or ≥50 EDs On-demand: treatment of acute bleeds; dose dependent on severity Part 3: Repeat pharmacokinetics OL, same patients as part 1 75 ± 5 IU/kg single-dose BAX326 after 26 ± 1 weeks of treatment	Characterize pharmacokinetics and establish equivalence with BFX (primary outcome = AUC _{0–72h}) Assess procoagulation markers (D-dimers, TAT, P1.2) Hemostatic efficacy (treatment and prophylaxis of bleeding) IR (assessed at each visit with 75 ± 5 IU/kg) ^a Safety (AEs, FIX inhibitors, antibodies, rFurin, CHO proteins, thrombotic events) HRQoL BAX326 PK parameters Re-evaluation of procoagulation markers	Windyga <i>et al.</i> [2014a, 2014c]
251101 (phase II/III) NCT01488994	Pediatric study (safety, efficacy, and pharmacokinetics in patients aged <12 years)	Age <12 years Severe/moderately severe disease Previously treated ^b Immunocompetent No history of FIX inhibitors	OL, R Two cohorts: age <6 years and 6 to <12 years Patients R within cohorts to either of two blood sampling sequences for PK assessment (75 ± 5 IU/kg single dose) Prophylaxis: 40–80 IU/kg twice weekly × ≥6 months or ≥50 EDs	Primary: safety (all AEs potentially related to BAX326) Secondary: pharmacokinetics, IR over time, hemostatic efficacy for 6 months, safety (immunogenicity, thrombotic events, laboratory data), HRQoL	Urasinski <i>et al.</i> [2013]; [ClinicalTrials. gov identifier: NCT01488994]

Continued

Table 1. Continued

Study number (phase) and ClinicalTrials.gov identifier	Study	Patients	Design and dosing	Objectives	Reference(s)
251001 (phase III) NCT01286779	Continuation study	Completed either the pivotal study or the pediatric study No history of FIX inhibitors	Treatment at the discretion of the investigator; twice weekly prophylaxis (50 IU/kg), modified prophylaxis, or on-demand	IR, efficacy, safety, immunogenicity, and HRQoL	[ClinicalTrials.gov, identifier: NCT01286779]
251002 (phase III) NCT01507896	BAX326 in patients undergoing surgical or other invasive procedures	Age 12–65 years Severe/moderately severe disease Requiring emergency or elective major or minor surgical, dental, or other invasive procedures	OL, NR <i>Preoperative:</i> BAX326 infusion to raise FIX to 80–100% for major surgery, and 30–60% for minor surgery <i>Postoperative:</i> dosing 8–24 hourly to maintain 80–100% FIX level, then 30–60% for 7 days after healing for major surgery; 24-hourly dosing to maintain 30–60% level for minor surgery	Primary: hemostatic efficacy and safety Secondary: intra- and postoperative hemostatic efficacy, intra- and postoperative blood loss, BAX326 and [AEs, FIX antibodies, thrombotic events], pharmacokinetics	Windyga <i>et al.</i> [2014b]; [ClinicalTrials.gov identifier: NCT01507896]
050901 (phase IV) NCT01128881	Immunine study	Age <65 years Severe/moderately severe disease No history of FIX inhibitors Immunocompetent	Immunine: 20–40 IU/kg twice weekly prophylaxis BAX326: as per pivotal and pediatric studies	Primary: prospective evaluation of pdFIX exposure and FIX inhibitors in patients planned for entry into the pivotal or pediatric study, hemostatic efficacy, IR (30 minutes), safety	Solano Trujillo <i>et al.</i> [2014] [ClinicalTrials.gov identifier: NCT01128881]

^aIncremental recovery (IR), defined as the peak factor level in plasma within the first hour of dosing expressed as the ratio of measured level against dose per bodyweight (IU/ml: IU/kg), is a key parameter used to characterize the pharmacokinetics of clotting factors.

^bAge ≥6 years: previously treated with pdFIX or rFIX for 150 EDs or participation in the Immunine Pre-Treatment Study (NCT01128881); age <6 years: previously treated with pdFIX and/or rFIX for >50 EDs or participation in the Immunine Pre-Treatment Study (NCT01128881).

AEs, adverse events; AUC_{0–72h}, area under the curve of plasma concentration *versus* time from 0 to 72 hours; BFX, nonacog alfa [BeneFIX]; CHO, Chinese hamster ovary; DB, double-blind; EDs, exposure days; FIX, factor IX; HRQoL, health-related quality of life; IR, incremental recovery; IU, international unit; NR, nonrandomized; OL, open-label; P1.2, prothrombin fragment 1.2; pdFIX, plasma-derived FIX; PK, pharmacokinetic; R, randomized; rFIX, recombinant FIX; rFurin, recombinant furin; TAT, thrombin-antithrombin III complex; WO, washout; XO, crossover.

Figure 2. Patient disposition (simplified) in the pivotal phase I/III study of BAX326 [Windyga *et al.* 2014a]. Parts 1 and 3 were single-dose pharmacokinetic (PK) evaluations; part 1 compared BAX326 and nonacog alfa in a crossover fashion, whereas part 3 was a repeat PK evaluation of BAX326 only. Part 2 was an open-label treatment and prophylaxis study of BAX326. See the paper by Windyga and colleagues [Windyga *et al.* 2014a] for full details of patient flow through the trial.

therapy, and a final repeat PK investigation (Table 1 and Figure 2). In total, 73 patients from 14 countries were enrolled into the full analysis set, of whom 59 received prophylaxis and 14 received on-demand treatment during the prophylaxis/treatment phase. At screening, most patients had arthropathy (88%) and target joints (66%), and only 18% were receiving consistent routine prophylaxis before the study [Windyga *et al.* 2014a]. A historic control group was generated by meta-analysis of 12 previously published studies. This was done due to the small size of the on-demand treatment group which was attributable to the increasing adoption of prophylaxis as the standard of care.

The study confirmed the PK equivalence of the 2 products based on area under the curve (AUC) of plasma concentration versus time from 0 to 72 hours (AUC_{0-72h}) [Windyga *et al.* 2014a]. Repeated PK analysis at the end of the 6-month study in the third part of the study confirmed the initial findings for BAX326. The incremental recovery at maximum concentration (C_{max}) was measured as 0.87 ± 0.22 and the half-life was 26.70 ± 9.55 hours, and 0.95 ± 0.25 IU/dl:IU/kg in the initial and final PK analyses. Moreover, the incremental recovery of BAX326 30 minutes postinfusion was consistent

over time, suggesting no formation of subclinical inhibitors [Windyga *et al.* 2014a].

Overall, compliance was very good among all 59 patients who received prophylactic treatment; 96.7% ($\pm 11.19\%$) of patients were in compliance with the determined prophylactic infusion dose (40–75 IU/kg), and 89.2% ($\pm 9.70\%$) were compliant with the planned frequency of dosing (any prior infusion was within 3.5 ± 1 days of the prophylactic infusion).

Of 56 patients who received prophylaxis for at least 6 months, 24 (42.9%) did not experience any bleeding. The overall median annualized bleeding rate (ABR) during the 6 months of twice-weekly prophylaxis with BAX326 was 1.99 (range 0–23.4). The overall mean ABR (4.20 ± 5.75) was significantly lower (79% difference; $p < 0.001$) than that seen in a historic control group of 276 patients who had received on-demand treatment (rFIX or pdFIX) in 12 earlier studies (20.0 ± 39.4) [Windyga *et al.* 2014a]. Of 249 reported bleeds in the pivotal study, 211 (84.7%) were controlled with 1–2 infusions. Hemostatic efficacy at bleed resolution was rated ‘excellent’ or ‘good’ in 96% of all bleeds (Figure 3) [Windyga *et al.* 2014a].

Figure 3. Patient rating of hemostatic efficacy at bleed resolution in the pivotal trial of BAX326 [Windyga *et al.* 2014a].

BAX326 was associated with a good safety profile in the pivotal study. There were no deaths, severe allergic reactions, or cases of anaphylaxis, thrombotic events, or treatment-related serious adverse events (AEs). No patient developed FIX inhibitors or treatment-related antibodies. Three non-serious AEs were considered related to BAX326 (two instances of dysgeusia in the same patient and pain in extremity in the other patient). Thrombogenic markers were elevated in some patients after treatment with either rFIX product, but were considered to be attributable to sampling technique or recent bleeding [Windyga *et al.* 2014a].

HRQoL was assessed using the Short Form (SF) 36 instrument administered at baseline and follow up. The SF-36 survey consists of 36 questions addressing physical and mental component summaries and 8 health domains, with higher scores indicating improvements [Maruish, 2011]. There were statistically significant and clinically meaningful (larger than the established minimally important difference for a given domain) improvements in patients on prophylaxis for the Physical Component Score of the SF-36 (mean change from baseline 2.60; $p=0.0189$) [Windyga *et al.* 2014a]. The improvement was due mainly to improvement in the domains of Bodily Pain and Role-Physical (ability to perform physical activities). Moreover, subgroup analysis showed that patients who switched to prophylaxis from on-demand treatment experienced more pronounced improvements across several domains: Physical

Component Score (mean change 3.21; $p=0.014$); Vitality (3.71; $p=0.04$); General Health (3.40; $p=0.009$); and Social Functioning (5.06; $p=0.002$). A post hoc analysis showed lower bodily pain in patients reporting zero bleeds ($p=0.038$) [Windyga *et al.* 2014c].

Pediatric study

The pediatric study (251101) (Table 1) [Urasinski *et al.* 2013; ClinicalTrials.gov identifier, NCT01488994] assessed safety, immunogenicity, pharmacokinetics and hemostatic efficacy during prophylaxis with BAX326 in previously treated patients aged <12 years with severe or moderately severe hemophilia B (FIX $\leq 2\%$). The primary endpoint was safety expressed in terms of AEs possibly or probably related to BAX326. The PK evaluation was based on a single infusion of BAX326 (75 ± 5 IU/kg) before the start of the prophylaxis period.

The study consisted of 23 children of median age 7.1 years, enrolled in 6 countries [Urasinski *et al.* 2013]. The majority of the patients (17/23, 73.9%) had FIX activity levels <1%, and 4 (17.4%, all aged ≥ 6 years) had arthropathy at screening. A total of 5 patients (21.7%, all aged ≥ 6 years) had at least 1 target joint [Urasinski *et al.* 2013].

BAX326 had a favorable safety profile in this population: no patients developed FIX inhibitors and there were no treatment-related anti-FIX or CHO

protein antibodies. No severe allergic reactions, no thrombotic events and no AEs related to treatment were reported. During the 6 months of prophylaxis, 39.1% (9/23) of patients had no bleeds and the median ABR for spontaneous bleeds was zero. Of a total of 26 bleeds, 23 were due to injury; 88.5% were treated with 1–2 infusions, and ‘excellent’ or ‘good’ hemostatic efficacy was reported in 96.2% of bleeds. Incremental recovery of BAX326 increased with age as expected (mean values $\pm$ standard deviation were 0.665 ± 0.1632 IU/dl:IU/kg in all patients, 0.586 ± 0.1320 IU/dl:IU/kg in children aged < 6 years, and 0.731 ± 0.1615 IU/dl:IU/kg in those aged 6 to < 12 years), and was consistent over the duration of the study [Urasinski *et al.* 2013].

Continuation study

The ongoing continuation study (251001) aims to investigate further incremental recovery over time, hemostatic efficacy, safety, immunogenicity and HRQoL of BAX326 in patients who have already completed the BAX326 pivotal study (250901) or pediatric study (251101), as well as in patients who have not received prior treatment with BAX326. Treatment is open-label and at the discretion of the investigator, and consists of twice-weekly prophylaxis with 50 IU/kg, modified prophylaxis or on-demand treatment [ClinicalTrials.gov identifier: NCT01286779].

Surgery study

The phase III surgery study (251002) [Windyga *et al.* 2014b; ClinicalTrials.gov identifier: NCT01507896] has been carried out to assess the safety and efficacy of BAX326 in patients undergoing surgical, dental, or other invasive procedures (Table 1). BAX326 was dosed according to whether patients were scheduled for major or minor surgery, with target FIX plasma levels of 80–100% relative to normal for major procedures and 30–60% for minor surgery (Table 1). Interim results for this study are available [Windyga *et al.* 2014b].

Of the 14 patients treated, 11 had major surgery and 3 had minor procedures. Of the 11 major surgeries, 7 were orthopedic (1 residual femur fracture nail removal, 5 joint replacements and 1 open synovectomy) and 4 were nonorthopedic (1 molar extraction, 2 hernioplastic procedures and 1 neurofibroma excision). Among the 3 minor surgeries, there were 2 dental procedures (first molar

extractions) and 1 intra-articular injection. Presurgical PK assessments were carried out in 7 patients. The mean FIX activity level was raised from 6.6% preoperatively to 107.6% after the initial loading dose for major procedures and from 3.6% to 81.4% for minor operations. Respective mean (range) weight-adjusted doses were 1265 (415–2965) IU/kg and 291 (55–601) IU/kg [Windyga *et al.* 2014b].

Intra-operative hemostatic efficacy was rated as ‘excellent’ for all procedures; postoperative efficacy at discharge was ‘excellent’ or ‘good’ for 11 and 3 patients, respectively. Mean intra-operative and postoperative blood losses were 236.8 ml and 784.4 ml, respectively. Actual intra-operative blood loss matched the predicted average blood loss in 5 major procedures, and was below prediction in all minor and 2 major procedures. Actual postoperative blood loss was at or below the predicted maximum in 3 major procedures. For the remaining 4 major operations, the actual loss was above the predicted maximum, but remained below 150% of the predicted maximum [Windyga *et al.* 2014b].

Mean presurgical PK parameters were available for 7 patients, among who the AUC_{0-72h} was 19.77 ± 7.88 IU/h/dl, incremental recovery (30 minutes) was 1.06 ± 0.35 IU/dl:IU/kg, and half-life was 21.50 ± 4.98 hours. There were no serious AEs, severe allergic reactions or thrombotic events, and no treatment-related antibody or FIX inhibitor formation. There were 14 nonserious AEs, but none of these were related to treatment with BAX326 [Windyga *et al.* 2014b].

Immunine switch study

This was the first prospective comparison of the safety, efficacy and incremental recovery of a pdFIX and rFIX in the same hemophilia B patients following a switch from the pdFIX Immunine to the rFIX BAX326. The patient population consisted of patients < 65 years of age with severe or moderately severe hemophilia B (FIX $\leq 2\%$) who completed a pretreatment study documenting exposure to Immunine and who subsequently transitioned into the pivotal or pediatric BAX326 studies (Table 1) [ClinicalTrials.gov identifier: NCT01128881; Solano Trujillo *et al.* 2014]. Immunine exposure and FIX inhibitor formation were monitored during prophylactic treatment over approximately 20–50 exposure days. The study also ensured that patients were

adequately pretreated and met the inclusion criterion for the pivotal or pediatric studies (150 exposure days for patients aged ≥ 6 years and 50 for patients aged < 6 years) [Solano Trujillo *et al.* 2014].

In total, 44 patients who completed prophylactic treatment with Immunine during the pretreatment study entered the pivotal and pediatric clinical studies. A total of 32 patients were aged ≥ 12 years (median 29.5 years) and subsequently entered the pivotal BAX326 study; 12 patients were aged < 12 years (median 7.5 years) and were later enrolled in the pediatric study (Table 2) [Solano Trujillo *et al.* 2014].

Overall, the transition from Immunine to BAX326 was clinically effective and safe. As in the other studies discussed in this review, no patients developed inhibitory or treatment-related FIX binding antibodies during treatment with either product or upon switching products. Safety profiles were similar for the two FIX products. There were no deaths, severe allergic reactions, anaphylactic reactions, thrombotic events or serious AEs related to treatment. There were 38 AEs (all unrelated to treatment) in 45.5% of patients during Immunine therapy. After switching to BAX326, there were 51 AEs in 56.8% of patients, only 2 of which (dysgeusia on 2 occasions in the same patient) were considered related to study treatment [Solano Trujillo *et al.* 2014].

Of the 32 patients who had incremental recovery data for Immunine, 13 (40.6%) did not experience any bleeds with this agent. Of all 44 patients who had incremental recovery data for BAX326, 21 (47.7%) had no bleeds. The ABR after ≥ 3 months of prophylaxis was slightly lower with BAX326 than with Immunine (mean 2.3 ± 3.3 versus 4.3 ± 7.6 in patients aged 12–64 years and 2.2 ± 2.38 versus 5.7 ± 7.33 in those aged < 12 years); the differences were not statistically significant. A higher ABR was observed for joint and spontaneous bleeds treated with Immunine (independent of baseline arthropathy) in patients aged ≥ 12 years (respective mean paired differences 2.2 and 1.0). This was likely attributable to these patients having received on-demand treatment before their entry into the pretreatment study, which resulted in a gradual ABR decrease during a period of transition and, in turn, resulted in a lower ABR over time (as observed in the BAX326 pivotal study [Windyga *et al.* 2014a]). Most bleeds showed an ‘excellent’ or ‘good’ response to either

Immunine or BAX326: 94.8% with BAX326 and 97.7% with Immunine [Solano Trujillo *et al.* 2014].

As expected, incremental recovery was lower for BAX326 than for Immunine in both age categories: median 0.89 versus 1.07 IU/dl:IU/kg for patients aged ≥ 12 years, and 0.67 versus 0.90 IU/dl:IU/kg for children aged < 12 years. This could be related to differences between pdFIX and rFIX in post-translational FIX polypeptide modification that may affect the PK properties of these agents [Ewenstein *et al.* 2002; White *et al.* 1997]. *In vivo* recovery appeared lower in children than in adolescents/adults, irrespective of treatment type. However, patient numbers were insufficient for definitive conclusions [Solano Trujillo *et al.* 2014].

Comments and conclusions

The development of the novel rFIX BAX326 is a significant step in the direction of increasing the global supply of rFIX and has the potential to reduce healthcare costs by introducing product competition. While many pdFIX products have been available for some time and viral inactivation and purification methods have improved considerably over the past two decades, rFIX products are not sourced from blood products and may offer additional safety advantages. The manufacturing procedure for BAX326, summarized briefly in this review, is designed to maximize product security in this respect.

Emerging data from the clinical study program for BAX326 show the safety and efficacy of the product in routine prophylaxis, in the management of acute bleeding episodes and in surgery, and after switching from another FIX product. The pivotal study has shown PK equivalence between BAX326 and nonacog alfa in addition to statistically and clinically significant improvements in control of bleeding relative to historic controls [Windyga *et al.* 2014a]. Moreover, BAX326 treatment was associated with significant improvements in HRQoL scores in this study; these improvements were seen across the full spectrum of patients, including those with pre-existing joint damage [Windyga *et al.* 2014a]. Early data obtained in children aged up to 12 years are similarly encouraging, with the large majority of bleeding episodes controlled with ‘excellent’ or ‘good’ efficacy, and median

Table 2. Patients and demographics in the phase IV Immunine study [Solano Trujillo *et al.* 2014].

Parameter	All patients treated with Immunine who subsequently entered the pivotal or pediatric BAX326 studies (n = 44)	
	Aged <12 years (n = 12)	Aged 12–64 years (n = 32)
Age, years		
Mean $\pm$ SD	6.4 $\pm$ 3.7	31.9 $\pm$ 11.4
Median (range)	7.5 (1–10)	29.5 (14–55)
FIX activity level, n (%)		
<1%	11 (91.7)	23 (71.9)
1–2%	1 (8.3)	9 (28.1)
Arthropathy at screening, n (%)		
Yes	4 (33.3)	26 (81.3)
No	8 (66.7)	6 (18.8)
FIX, factor IX; SD, standard deviation.		

ABR of zero for spontaneous bleeds. Interim results from the phase III surgery study show consistent high hemostatic efficacy for patients undergoing invasive procedures [Windyga *et al.* 2014b].

Most recently, results have become available from the first prospective comparison of a pdFIX and rFIX in the same group of patients with hemophilia B after switching from Immunine to BAX326. Safety considerations have been a concern for patients who switch products because of the potential for the formation of neoantigens, but there is a consensus in the literature that the overall risk of inhibitor formation is low for hemophilia B patients when switching FIX products [Matino *et al.* 2014]. Data are fewer than for patients with hemophilia A, but this is partly because of the lower incidence of hemophilia B relative to hemophilia A, and the lower frequency of inhibitor formation in hemophilia B [DiMichele, 2012]. The emerging results of the Immunine switching study align with these observations [Solano Trujillo *et al.* 2014]. None of the 44 patients who transitioned from Immunine to BAX326 developed inhibitory or specific binding antibodies, which implies that differences in protein cleavage and pharmacokinetics do not affect any potential antigenicity of either product. In clinical studies to date, no FIX inhibitors have been observed in relation to treatment with

BAX326 [Warrier *et al.* 1997; Roth *et al.* 2001; Poon *et al.* 2002].

Overall, safety results in the clinical study program to date have been strong. There have been no serious AEs, no severe allergic reactions or thrombotic events associated with BAX326, and the large majority of nonserious AEs have been unrelated to study treatment.

Studies continue to show that the incremental recovery of BAX326 is lower than that of pdFIX, in accordance with earlier data comparing rFIX with pdFIX products [Ewenstein *et al.* 2002; Poon *et al.* 2002]. Lower recoveries noted in younger patients are thought to relate to the higher plasma volume seen in young children relative to older persons [Poon *et al.* 2002].

In conclusion, rFIX products in general have shown excellent safety and efficacy profiles in patients with hemophilia B. Accumulating data for BAX326 continue to show this pattern, with significant HRQoL benefits and no evidence to date of inhibitor formation, anaphylaxis or specific antibody formation. Ongoing work, such as the continuation study mentioned in this review, will continue to provide further clinical and safety data.

Conflict of interest statement

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