EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

23 October 2014
EMA/702760/2014
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Rixubis

International non-proprietary name: nonacog gamma

Procedure No. EMEA/H/C/003771/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

Table of contents

1. Background information on the procedure 4
1.1. Submission of the dossier 4
1.2. Manufacturers 5
1.3. Steps taken for the assessment of the product 5
2. Scientific discussion 6
2.1. Introduction 6
2.2. Quality aspects 8
2.2.1. Introduction 8
2.2.2. Active Substance 8
2.2.3. Finished Medicinal Product 13
2.2.4. Discussion on chemical, pharmaceutical and biological aspects 18
2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects 18
2.2.6. Recommendations for future quality development 18
2.3. Non-clinical aspects 19
2.3.1. Introduction 19
2.3.2. Pharmacology 19
2.3.3. Pharmacokinetics 21
2.3.4. Toxicology 23
2.3.5. Ecotoxicity/environmental risk assessment 31
2.3.6. Discussion on non-clinical aspects 31
2.3.7. Conclusion on the non-clinical aspects 34
2.4. Clinical aspects 34
2.4.1. Introduction 34
2.4.2. Pharmacokinetics 36
2.4.3. Pharmacodynamics 45
2.4.4. Discussion on clinical pharmacology 45
2.4.5. Conclusions on clinical pharmacology 47
2.5. Clinical efficacy 47
2.5.1. Dose response studies 47
2.5.2. Main studies 47
2.5.3. Discussion on clinical efficacy 83
2.5.4. Conclusions on the clinical efficacy 86
2.6. Clinical safety 86
2.6.1. Discussion on clinical safety 91
2.6.2. Conclusions on the clinical safety 94
2.7. Pharmacovigilance 94
2.8. Risk Management Plan 94
2.9. User consultation 99
3. Benefit-Risk Balance 100
4. Recommendations 102

List of abbreviations

AEs	Adverse Events
Ag	Antigen
aPTT/ APTT	Activated Partial Thromboplastin time
AUC	Area under the curve
BAX 326	Baxter's recombinant factor IX (company code for Rixubis drug substance)
Cl	Total clearance
Cmax	Maximum Concentration
ECB	Evaluation Cell Bank
ECG	Electrocardiogram
ELISA	Enzym-Linked ImmunoSorbent Assay
EUHASS	European Hamophilia Safety Surveillance
FIX	Factor IX
FIXa	Activated Factor IX
GLP	Good Laboratory Practice
IP	Investigational Product
IR	Incremental Recovery
IU	International Units
IVR	In Vivo Recovery
ko	Knock-out
LLOQ	Lower Limit of Quantification
MRT	Mean Residence Time
n.a.	Not applicable
NOAEL	No observed adverse effect level
NZW	New Zealand White
p	p-value of Probability
pdFIX	Plasma-derived FIX
PedNet	Paediatric Network
PK	Pharmacokinetic(s)
PLT	Platelet Count
PMCB	Premaster Cell BAnk
PT	Prothrombin Time
PUPs	Previously untreated patients
rFIX	Recombinant factor IX
Vss	Volume of Distribution at steady state

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Baxter Innovations GmbH submitted on 28 October 2013 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Rixubis, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The applicant applied for the following indication:

Treatment and prophylaxis of bleeding in patients with haemophilia B (congenital factor IX deficiency)

Rixubis is indicated in patients of all age groups.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated nonacog gamma was considered to be a known active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on the applicants' own tests and studies and/or bibliographic literature substituting/supporting certain tests or studies.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0159/2012 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0159/2012 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

New active Substance status

N/A

Scientific Advice

The applicant received Scientific Advice from the CHMP on 18 March 2010. The Scientific Advice pertained to quality and clinical aspects of the dossier.

Licensing status

Rixubis has been given a Marketing Authorisation in USA on 27.06.2013.

A new application was filed in the following countries: Australia, Switzerland, Colombia, New Zealand, Democratic People's Republic of Korea and Canada.

1.2. Manufacturers

Name and address of the manufacturer of the biological active substance

Baxter AG
Uferstraße 15
A-2304 Orth an der Donau
Austria

Name and address of the manufacturer responsible for batch release

Baxter SA
Boulevard René Branquart 80
B-7860 Lessines
Belgium

1.3. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were :

Rapporteur: Andrea Laslop Co-Rapporteur: Pierre Demolis

CHMP Peer reviewer: Ondřej Slanař

- The application was received by the EMA on 28 October 2013.
- The procedure started on 20 November 2013.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 10 February 2014. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 7 February 2014.
- During the meeting on 20 March 2014, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 20 March 2014.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 26 May 2014.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 30 June 2014.
- During the CHMP meeting on 24 July 2014, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 21 August 2014.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 02 September 2014.
- During the CHMP meeting on 25 September 2014, the CHMP agreed on a 2nd list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP 2nd List of Outstanding Issues on 01 October 2014.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of

Questions to all CHMP members on 09 October 2014.

- During a meeting of the Biologics Working Party on 13-15 October 2014, experts were convened to address questions raised by the CHMP.
- During the meeting on 23 October 2014, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Rixubis.

2. Scientific discussion

2.1. Introduction

Problem statement

Haemophilia B

Haemophilia B, or congenital Factor IX (FIX) deficiency, is an X-chromosomal-linked bleeding disorder with an incidence of approximately 1 in 30,000 live male births. Haemophilia B is the second most common type of haemophilia and is 5 times rarer than haemophilia A. The World Federation of Haemophilia (WFH) reported a worldwide prevalence of 399,000 subjects with haemophilia, of which there are an estimated 80,000 patients with haemophilia B. In approximately 30 percent of haemophilia B cases, there is no family history of the disorder and the condition is the result of a spontaneous gene mutation.

FIX is a vitamin-K-dependent coagulation factor that belongs to the class of serine proteases. FIX participates in the intrinsic pathway of blood coagulation by activating factor X to its active form in the presence of Ca2+ ions, phospholipids, and cofactor VIIIa (tenase complex). The synthesis of FIX takes place in the hepatocytes, from where it is secreted into the circulation. The physiological plasma concentration of FIX is 1 IU/mL, which equals about 5 µg/mL. The plasma levels of FIX determine the severity of the disease, which is classified in severe (< 1% of normal), moderate (1 to <5% of normal) or mild haemophilia B (> 5% of normal).

Severely diminished or absent levels of circulating FIX in haemophilia B result from impaired synthesis caused by major defects in gene structure (large deletions, insertions, frameshifts, and nonsense mutations) or from the rapid destruction of unstable defective FIX molecules. In contrast, missense mutations are often associated with aberrantly expressed FIX molecules that can be detected immunologically (cross-reactive material [CRM(+)]) but exhibit reduced activity in coagulation-based assays. Historically, haemophilia patients were only treated when they had bleeding episodes (on-demand). One of the main reasons on-demand treatment is used is due to the high cost and limited supply of FIX products. However, it has become known that treatment of severe haemophilia with frequent, periodic prophylactic FIX infusions can have significant medical and quality of life benefits. On prophylaxis, adequate plasma levels of FIX for haemostasis are maintained, approximating a non-diseased state. Prophylaxis treatment started at a young age would facilitate a complete lack of bleeding episodes and help to maintain healthy joints.

About the product

The active substance of Rixubis is constituted of purified FIX protein produced by recombinant DNA technology. The drug product is formulated as a sterile, nonpyrogenic, lyophilized powder preparation, intended for intravenous (IV) infusion. It is available in single-use vials containing the labelled amount of factor IX activity, expressed in International Units (IU). Each vial contains nominally 250, 500, 1000, 2000, or 3000 IU of coagulation factor IX (recombinant). After reconstitution, the solution is clear,

colourless, free from foreign particles and has a pH of 6.8 to 7.2. The osmolality is greater than 240 m osmol/kg.

Rixubis contains recombinant coagulation factor IX (nonacog gamma). Factor IX is a single chain glycoprotein with a molecular mass of about 68,000 Dalton. It is a vitamin K-dependent coagulation factor and it is synthesised in the liver. Factor IX is activated by factor XIa in the intrinsic coagulation pathway and by factor VII/tissue factor complex in the extrinsic pathway. Activated factor IX, in combination with activated factor VIII, activates factor X. Activated factor X converts prothrombin into thrombin. Thrombin then converts fibrinogen into fibrin and a clot is formed.

The Applicant applied for the following indication which was finally approved: Rixubis is indicated for the treatment and prophylaxis of bleeding in patients with haemophilia B (congenital factor IX deficiency). Rixubis is indicated in patients of all age groups.

The dose and duration of the substitution therapy depends on the severity of the factor IX deficiency, on the location and extent of the bleeding, and on the patient's clinical condition, age and pharmacokinetic parameters of factor IX, such as incremental recovery and half-life.

During the course of treatment, appropriate determination of factor IX levels is advised to guide the dose to be administered and the frequency of repeated infusions. Individual patients may vary in their response to factor IX, demonstrating different half-lives and recoveries. Dose based on bodyweight may require adjustment in underweight or overweight patients. In the case of major surgical interventions in particular, precise monitoring of the substitution therapy by means of coagulation analysis (plasma factor IX activity) is indispensable.

To ensure that the desired factor IX activity plasma level has been attained, careful monitoring using an appropriate factor IX activity assay is advised and, if necessary, appropriate adjustments to the dose and the frequency of repeated infusions should be performed. When using an in vitro thromboplastin time (aPTT)-based one stage clotting assay for determining factor IX activity in patients' blood samples, plasma factor IX activity results can be significantly affected by both the type of aPTT reagent and the reference standard used in the assay. This is of importance particularly when changing the laboratory and/or reagents used in the assay.

The number of units of factor IX administered is expressed in International Units (IU), which are related to the current WHO standard for factor IX products. Factor IX activity in plasma is expressed either as a percentage (relative to normal human plasma) or in International Units (relative to an International Standard for factor IX in plasma).

The calculation of the required dose of factor IX for "On demand treatment" in adults is based on the empirical finding that 1 International Unit (IU) factor IX per kg body weight raises the plasma factor IX activity by 0.9 IU/dL (range from 0.5 to 1.4 IU/dL) or 0.9% of normal activity in patients 12 years and older (further information see SmPC sections 4.2; 5.2). In the paediatric population the calculation of the required dose of factor IX for on demand treatment is based on the empirical finding that 1 International Unit (IU) factor IX per kg body weight raises the plasma factor IX activity by 0.7 IU/dL (range from 0.31 to 1.0 IU/dL) or 0.7% of normal activity in patients less than 12 years of age (further information see section 5.2).

The required dose is determined using the following formula:

Patients 12 years and older

Required units	=	body weight (kg)	x	desired factor IX rise (%) or (IU/dL)	x	reciprocal of observed recovery (dL/kg)

For an incremental recovery of 0.9 IU/dL per IU/kg, the dose is calculated as follows:

Required units	=	body weight (kg)	x	desired factor IX rise (%) or (IU/dL)	x	1.1 dL/kg

For long-term prophylaxis against bleeding in patients with severe haemophilia B, the usual doses are 40 to 60 IU of factor IX per kilogram of body weight at intervals of 3 to 4 days for patients 12 years and older. In some cases, depending upon the individual patient´s pharmacokinetics, age, bleeding phenotype and physical activity, shorter dosage intervals or higher doses may be necessary.

For an incremental recovery of 0.7 IU/dL per IU/kg in paediatric patients <12 years, the dose is calculated as follows:

Required units	=	body weight (kg)	x	desired factor IX rise (%) or (IU/dL)	x	1.4 dL/kg

The recommended dose range for prophylaxis in paediatric patients less than 12 years is 40 to 80 IU/kg at intervals of 3 to 4 days. In some cases, depending upon the individual patient´s pharmacokinetics, age, bleeding phenotype and physical activity, shorter dosage intervals or higher doses may be necessary.

Rixubis is administered intravenously up to a maximum of 10 ml/min.

2.2. Quality aspects

2.2.1. Introduction

Rixubis is a recombinant factor IX clotting factor. It has structural and functional characteristics comparable to those of endogenous FIX and to the approved rFIX product, Benefix.

The active substance is produced and secreted by a FIX/furin co-expressing CHO cell line where the Furin endopeptidase will cleave signal peptide and propeptide (pro-FIX) sequences.

No monoclonal antibodies, no materials of human or animal origin are employed in the manufacture, purification, or formulation of the final product, thus reducing the risk of transmission of adventitious agents. The process includes solvent/detergent treatment and 15 nm nanofiltration as virus inactivation/removal steps.

The drug product is presented as a powder and solvent for injection (IV use) in 5 dosage strengths 250, 500, 1000, 2000 and 3000 IU/vial. All dosage strengths are formulated with 20 mM L-histidine, 60 mM sodium chloride, 4 mM calcium chloride, 110 mM mannitol, 35 mM sucrose and 0.005% wt polysorbate 80 and are reconstituted with 5 ml water for injections.

2.2.2. Active Substance

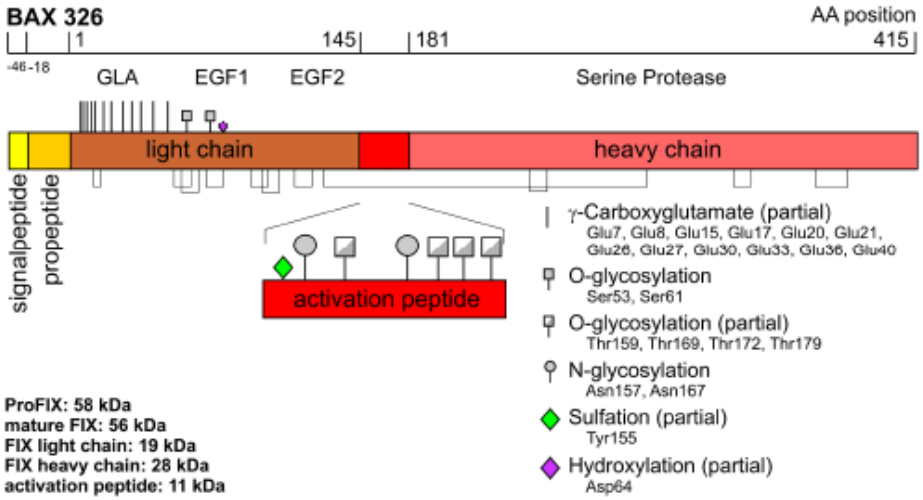
General Information

Sufficient general information, including nomenclature, structural formula and general properties has been provided. The company code is BAX 326.

Rixubis rFIX is a glycoprotein which is secreted by a genetically engineered FIX/furin co-expressing CHO cell line. It has 415 amino acids in a single chain corresponding to the mature zymogen and the primary amino acid sequence is comparable to the Ala148 allelic form of plasma–derived factor IX, however, some post-translational modifications of the recombinant molecule are different to those of the plasma-derived

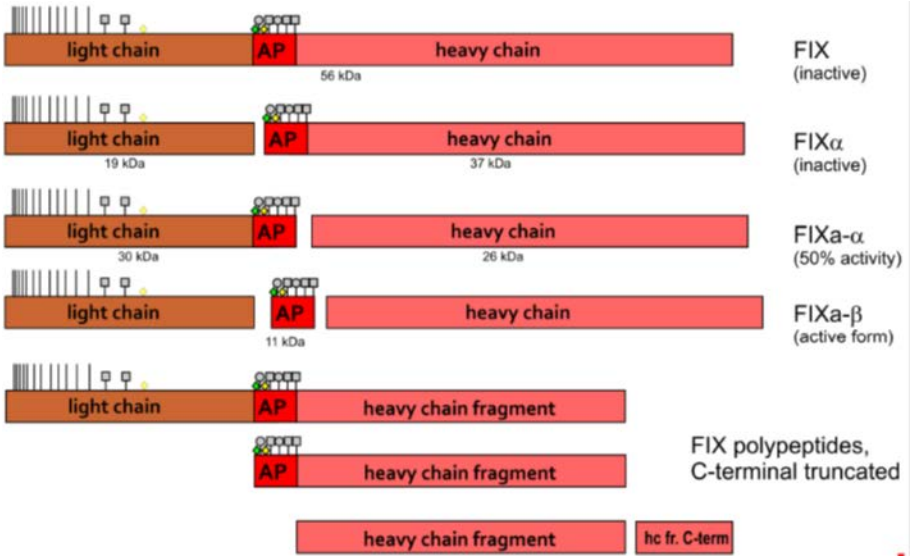
molecule. It is synthesized as a single chain polypeptide and secreted in its mature form. The molecule is folded in a 3-dimensional structure and consists of several discrete functional domains, including the Gla domain, two epidermal growth factor domains (EGF1 and EGF2), an activation peptide and the catalytic domain. As in other vitamin K-dependent proteins, the Rixubis rFIX undergoes a number of post-translational maturation events prior to secretion such as gamma-carboxylation, N- and O glycosylation, sulfation and hydroxylation. These modifications are located in light chain and activation peptide. No post translational modification is observed on heavy chain.

Figure: Schematic overview of the Rixubis Drug Substance molecule (Company code BAX326):



Activation of factor IX occurs by cleavage after Arg145 and Arg180 by the action of factor XIa (intrinsic coagulation pathway) or factor VIIa/tissue factor (TF) (extrinsic coagulation pathway) which results in two disulfide-linked peptide chains (light chain and heavy chain). Incomplete cleavage can result in partially activated forms of factor IX as shown below:

Figure: Schematic overview of FIX activation peptides



Manufacture, characterisation and process control

Manufacturing sites

All sites were inspected by European competent authorities within the 3 last years and were deemed compliant with European GMP Guidance for the manufacture of the active substance.

Description of the manufacturing process and process controls

Overall, the active substance manufacturing process is considered as adequately described. It consist of 11 manufacturing steps including continuous cell culture, purification by anion and affinity chromatography, two virus inactivation/removal steps, concentration and pre-formulation steps. Flow charts have been presented. The ranges of critical process parameters and routine in-process controls along with acceptance criteria are described for each step and are considered adequately set to control the manufacturing process. The active substance manufacturing process is considered acceptable.

Control of materials

Suppliers of raw materials are selected based upon their ability to supply materials that consistently meet specifications established for each raw material used in the manufacture of rFIX. Compendial materials comply with the specified pharmacopoeia monographs. Compendial and non-compendial raw materials are tested either by their manufacturer, by Baxter, or by contractors except identity testing. Identity testing is carried out in Baxter laboratories.

Fetal bovine serum (FBS) originated from countries with a controlled BSE risk was used in the development of the MCB. No other human or animal-derived materials were used upstream of the MCB. Furthermore, no proteins of human or animal origin are used in the commercial manufacturing process starting from the master cell bank.

The cDNA encoding human factor IX carrying the Ala148 polymorphism was originally isolated from a randomly primed human liver cDNA library. A stably transfected CHO cell line expressing factor IX and Furin was generated. The construction of the expression vector is adequately described. Sufficient information has been provided regarding the characteristics of the plasmid and the producer cell line.

The Working Cell Bank (WCB) used to manufacture rFIX Bulk Drug Substance (BDS) is derived from the Master Cell Bank (MCB) for cell line CHO rFIX. Acceptable information has been submitted regarding the testing of MCB, WCB and EOP and release of future WCBs. Genetic stability has been demonstrated for cells at and beyond the limit of cell age.

It has been confirmed that in case of a change to the cell banking, a variation will be submitted.

Process validation

Process validation was performed at the commercial scale manufacturing facility. It should be noted that the DS manufacturing process was studied through a Design of Experiments (DOEs) approach during Pharmaceutical development; however, no design space is claimed.

To get a clear understanding of the process description with its control strategy, the applicant submitted, as requested at D180, a comprehensive list of critical process parameters for each DS manufacturing step in a tabular format. For each CPP, the set points and ranges have been specified, when appropriate. In the updated description of the process some non CPPs which generally correspond to PP that are usually found under the description of the process are mentioned.

An integrated approach to process validation was taken. The consistency and robustness of full scale manufacturing was demonstrated through process verification carried out with the conformance lots. The life-cycle of the process is managed through a process monitoring program.

Process validation was performed to demonstrate the following:

- Manufacturing process control and robustness
- intra-production run consistency (all batches within a given production run)
- inter-production run consistency (batches from start, middle and end of several production runs)
- manufacturing process capability
- impurity removal (inter-production run capability).

Data collected during process validation showed that the process can consistently manufacture rFIX product which meets the product specifications. The rFIX production process yields a high purity product with a high rFIX specific activity and a low aggregate and dimer content. Extended characterisation provides support for the consistency of the manufacturing process within and between the production runs. No differences were observed between the beginning, middle and end of a production run, and extension of a production run and extended hold times did not impact the characteristics of rFIX product. The data collected during process validation show that the process is capable of reducing process related impurities and product related impurities. Some discrepancies regarding the specification limits and the conformance batches used for process validation were identified and clarified during the procedure. Concerns originating from the reported rFIX specific activity values (U/mg) as measure of purification efficacy for each purification step have been solved.

Process validation data of 3 different end runs have demonstrated that the chaemostat run time has now been sufficiently validated.

Manufacturing process development

The manufacturing process was scaled up in 3 stages . A chronological summary of the process modifications is presented, in relation to the production runs and DS batches concerned. The downstream production runs (and related DS batches) could be considered as obtained according to a representative process of the commercial process. The related DS batches were studied through process validation, characterisation and stability. Regarding the corresponding DP batches, they were also studied through validation, characterization and stability of the DP. On a quality aspect, the recent and representative versions of the manufacturing process have been adequately studied.

On a clinical aspect, it is noted that representative DP batches were studied during Pivotal Phase I-III clinical studies 251002 and 251101, while the Pivotal clinical study 250901 involved batches issued from previous and less representative manufacturing processes. It could be noted that DP batches from the production run, which corresponds to the last version of the manufacturing process, were not used during these Pivotal clinical studies. However, within the response to D180 LOI, the applicant provided a comparability study on quality aspects, between DP batches used in pivotal clinical studies, obtained with a former production and the commercial DS process. The comparability study has considered the DP release tests. Results are comparable.

In addition, a comprehensive characterization comparability study has been provided (in response to a non-clinical question), which also demonstrates the comparability between non-clinical, early clinical and clinical / commercial batches.

The rFIX one-stage clotting assay used for product labelling was improved during product development; however, pieces of information on the improvement of the method and the date of implementation were

not given. Furthermore, it appears that the Phase III clinical batches were tested according to the rFIX clotting assay before being improved. The applicant has provided data from the study leading to the improvement of the rFIX one-stage clotting assay during the procedure.

In the EMA/EDQM Workshop *Characterisation of new clotting factor concentrates (FVIII, FIX) with respect to potency assays used for labelling and testing of post infusion samples*, held on 28-29 November 2013, the applicant has indicated that potency results can be affected by the type of aPTT reagent used in the clotting assay. As requested in the Day180 LoOI further data on the aPTT dependency of the clotting assay have been provided. A study report which summarizes the investigations performed by the Company has been submitted as part of the MAA for Rixubis: rFIX activities of Rixubis and BeneFIX were determined with the 1-stage clotting assay using 12 different aPTT reagents, the resulting potency was calculated relative to the labelled potency. In general higher recoveries were found for Rixubis (87% - 144%) while for BeneFIX values between 64% and 111% were measured. The Dapttin reagent, for which a value of 98% has been measured, will be used as basis for the labelled potency of Rixubis. Concerning the possible consequences of the dependency of assay results on the aPTT reagent on product information the applicable changes of the revised core SmPC for FIX products have been implemented which includes high level information that plasma factor IX activity results can be significantly affected by both the type of aPTT reagent and the reference standard used in the assay.

Characterisation

The rFIX active substance has been sufficiently characterised by physicochemical and biological state-of-the-art methods. rFIX was investigated by a number of analytical methods focusing on the elucidation of the primary structure, post-translational modifications, three dimensional structures and the biological activity. A selected set of conformance Drug Substance samples representing the beginning, middle and end of three campaigns, as well as samples representing the longest possible hold times were used. Furthermore clinical Drug Product samples representing different potencies and samples from a marketed, rFIX comparator product with different potencies were included in this characterisation evaluation.

Conformance batches of Rixubis (Drug Substance and Drug Product) were characterised with respect to their haemostatic potency, the efficiency of activation by FXIa and FVIIa in the presence of tissue factor and their capacity to bind to phospholipid vesicles. In addition, the functional properties of Rixubis were compared to three batches of the licensed, marketed, recombinant factor IX product with different potencies.

A number of process and product related impurities were identified. Process-related impurities include biological impurities, chemical impurities, and other impurities from the fermentation medium. Product-related impurities derive from a number of modifications of rFIX such as aggregation, fragmentation, oxidation and deamidation.

Impurities are controlled via appropriate specifications at Drug Substance level and/or their clearance during downstream-processing has been demonstrated through process validation. In summary, all impurities were found to be consistent within non-clinical and clinical Drug Substance production. Furthermore, consistency between different phases of fermentation (begin, middle, end) and different production runs could be shown. For some of the process-related impurities, for which clearance has been addressed in the process validation part, a description of the used analytical method has been provided during the procedure. Also additional information concerning the truncated forms and the clotting assay has been submitted. Information on the content of gamma carboxylation of N-terminal glutamic acid was provided with the response to the D180 LoI. It is recommended as committed that additional studies on the characterisation of charged active substance variants by IEF will be performed post authorisation (see section 2.2.6).

Specification

Specifications for the drug substance were set in accordance to guideline ICH Q6B. The drug substance is routinely controlled by a range of chemical-physical and biological tests to assure consistent production of the drug substance.

The initially proposed strategy for control of the glycosylation profile, which was not acceptable, has been revised and is now considered as appropriate. Also for the SDS-PAGE under reduced and non-activated conditions an acceptable justification for its use as identity test only has been provided. A justification for the Drug Substance specifications as well as the batch numbers for set-up of each DS acceptance criterion has been submitted. The requested tightening of the acceptance criteria for the HCP limits has been implemented and this issue is now solved.

Analytical methods

A description of the test methods utilised for the release analysis of rFIX Drug Substance has been submitted, the validations of the analytical procedures were performed in conformance with the USP and European Pharmacopoeia guidelines. Details of the validation of analytical procedures and the SEC-HPLC method have been provided and updated during the procedure.

For reference standards and materials reference is made to the Drug Product section.

Container closure system

The container closure system used for the storage of Rixubis BDS is the Nalgene square media bottle 1000 mL. The bottle body is made of polyethylene terephthalate copolyester (PETG) and the screw cap is made of high density polyethylene (HDPE). Both the bottle body and the screw cap are considered product contact surfaces. Compliance of the container closure system with relevant Ph. Eur. monographs has been confirmed as well as a discussion on potential leachables/extractables has been provided.

Stability

The submitted stability data support the proposed shelf life (24 months) and storage conditions of the active substance (≤-60° C). Stability data were provided for eight commercial scale drug substance batches (3 clinical batches and 5 conformance lots). For the stability studies, samples were directly filled into 30 mL sterile PETG vials prior to freezing. The filling volume is 2.0 mL, representing a worst case surface-to-volume ratio. All vials were stored in the upright orientation. The parameters used to monitor the stability of the rFIX Drug Substance were selected to measure the quality, purity and potency of the rFIX Drug Substance. The parameters and specification used in the stability studies are a subset of the rFIX Drug Substance release parameters. The proposed testing strategy for product-related impurities during stability testing will be updated with respect to the tightening of molecular size distribution limits and the addition of a control test of the glycosylation pattern.

2.2.3. Finished Medicinal Product

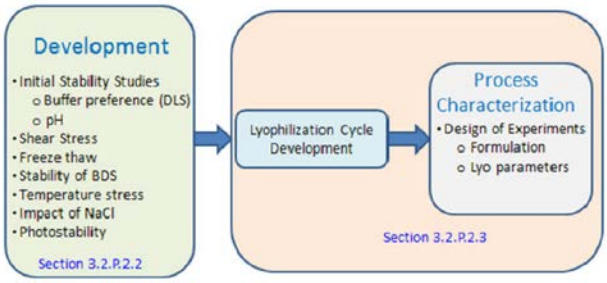
Description of the product and pharmaceutical development

Description and composition

The Rixubis Final Drug Product (FDP) consists of a lyophilized powder for solution for injection and is supplied in a single-dose glass vial. The proposed nominal dosage strengths are 250, 500, 1000, 2000 and 3000 IU/vial. All dosage strengths are formulated with L-histidine, sodium chloride, calcium chloride, mannitol, sucrose and polysorbate 80. Each dosage strength is reconstituted with a nominal volume of 5 mL sterile Water for Injection (sWFI) and mixed prior to intravenous injection. Rixubis is packaged into a product kit consisting of one product vial, one diluent vial containing approximately 5 mL of sterile Water for Injection (sWFI), one BaxJect II needleless transfer device, and one package insert (PI).

Pharmaceutical Development

The Rixubis Drug Product process development took a systematic, stepwise approach. The three steps, as outlined below were initial screening of conditions and buffer components for product compatibility and stability, including thermal and photo stability. Subsequently, the critical lyophilisation process parameters were studied and the final manufacturing process was defined. As a last step, the finalised manufacturing process parameters (target and ranges) were characterised using a DoE approach. Since the formulation and lyophilisation processes are interdependent, an integrated approach to process characterisation was utilised.



A detailed overview of the correspondences between DS manufacturing processes, DP manufacturing processes, related DS production runs, DS and DP batches as well as use of these different batches in Preclinical and Clinical studies has been provided.

The formulation development took a systemic approach starting with screening for the impact of the pH, investigations on the buffer preference, and the impact of shear stress. The development work included also freeze-thaw studies, photo- and thermal stability studies; the influence of calcium on stability of Rixubis in liquid formulations as well as the influence of sugars on the aggregation was evaluated. Optimisation of the formulation included also an increase of the NaCl concentration in order to receive a better lyophilisation cake appearance while maintaining good product stability. No formulation overages are used for the Rixubis Drug Product. The nominal fill weight for each vial includes an overage intended to allow for fill variances, thereby ensuring that a full dose is provided upon reconstitution.

Lyophilisation process development started with characterisation of the freeze-drying behaviour of the formulation buffer for rFIX by low temperature thermal analysis and by freeze dry microscopy. These results were compared with results of trial freeze dry cycles carried out under increasingly aggressive conditions until visual collapse was observed in the final freeze-dried product. Additionally, the vial heat transfer coefficient vs. pressure, the resistance of the dried product layer to flow of water vapour, and the equipment capability curve was used to construct a design space for primary drying.

The final sterile filtration step and the corresponding process parameters were evaluated in terms of capacity, and influence on the product quality.

A DoE approach was utilised to evaluate the influence of the variation of various process parameters of the existing formulation and lyophilisation process on the quality attributes of the rFIX FDP. Eight formulation parameters (six excipient concentrations, pH of formulated bulk, and rFIX clotting activity of formulated bulk) coupled with three lyophilisation parameters (freezing ramp rate/annealing time, annealing temperature and chamber pressure) were used as a variable for DoEs.

In summary, reconstitution time, residual moisture and rFIX pre-activation activity are barely affected by the parameter variations of the DoE. The highest influence on quality attributes are

aggregation/dimerization, the loss of rFIX clotting activity and specific activity. However, none of the DoE parameter settings result in final containers with an average dimerization of more than 1.0% and an average aggregation of more than 0.5%, which is far below the specification limit of 2.0%. The maximum loss of rFIX clotting activity is never more than 15%. The minimum observed recovery of rFIX specific activity is 73%.

Manufacture of the product and process control

The drug product manufacturing process has been adequately described. Appropriate in-process controls have been identified and are monitored to control the manufacture of rFIX Drug Product. In-process controls, test methods, as well as actions to be taken if out of range are presented.

Briefly, the drug product manufacturing process comprises of the following steps: formulation, sterile filtration and filling, lyophilisation, capping, air ingress testing, visual inspection, packaging and labelling.

Validation and evaluation studies were performed to demonstrate manufacturing process control, robustness and consistency. In summary, acceptance criteria were established in each validation protocol and results passed the acceptance criteria or were properly investigated. Based on the validation reports, it was concluded that the process and equipment are suitable for consistent manufacturing of Rixubis drug product.

Control of excipients

The excipients, L-Histidine, Calcium Chloride, Sodium Chloride, Mannitol, Tween 80 (Polysorbate 80), Sucrose and WfI are described in Pharmacopoeias and are tested to meet the requirements of Ph. Eur., USP and JP as available. The validity of bacterial Endotoxin and Bioburden assays was verified for each excipient.

Product specification

In accordance with guideline ICH Q6B, specifications were set to support the release of rFIX drug product and are found suitable for its control.

Questions related to the control of product-related impurities at Drug Product level have been appropriately addressed and solved. Also the tightening of the molecular size distribution limits for monomer, dimer and aggregates is considered acceptable. Additional information regarding the measurement of pro-FIX content has been submitted as requested. Observed discrepancies regarding the specification range for potency have been clarified.

A description of the test methods utilised for the release analysis of rFIX Drug Product has been submitted, the validations of the analytical procedures were performed in conformance with the USP and European Pharmacopoeia guidelines. Details of the validation of analytical procedures have been provided.

An improved rFIX activity method (clotting assay) has been implemented in response to detected OOS values during stability testing. The applicant gives information about the improvements which were implemented in the assay: in principal the mentioned points for an improvement of such a test is acceptable. In addition Baxter commits to investigate a further improvement of the FIX clotting assay, and, if necessary, will come back to the authorities 1 year after Commission Decision. In case of changes to the potency method, a variation for approval will be filed post-authorisation (see section 2.2.6).

A summary of batch analysis data of 15 lots of rFIX Drug Product (3 lots of 250 IU, 3 lots of 500 IU, 4 lots of 1000 IU, 2 lots of 2000 IU, and 3 lots of 3000 IU) is presented in the dossier and demonstrate consistency of the manufacturing process.

Reference standards or materials

An in-house rFIX reference standard (lot HP02R) has been established from a drug substance lot to measure the potency of rFIX Drug Product. The potency of the rFIX in-house standard (85 IU/mL) was assigned by one-stage clotting method using WHO 4th IS FIX Concentrate. In addition, a comparative study was also conducted to establish a statistical value for the average percent difference in potency due to the switch from the current plasma-derived in-house potency standard to the rFIX in-house potency standard by the same method. The stability of the rFIX reference standard was evaluated under accelerated degradation conditions to calculate its real time stability at ≤-20° C. The real time stability of this reference standard will be monitored for 7 years. The Company has submitted real time/real temperature data (to justify the approach for determination of the real time shelf-life and to clarify other, initially identified discrepancies. As requested specific protocols for establishment of a new reference material / standard have been provided and updated with respect to the N-glycan content determination taking the revised control strategy for the differently charged oligosaccharides into account. Authorities will be informed if an OOS event should occur and a new rFIX standard will be established in this case.

Container closure system

The container closure system for the drug product has been adequately described and complies with the requirements of the European Pharmacopoeia (Ph. Eur.). All dosage strengths are filled into 10 ml vials made of neutral glass, hydrolytic Type I. The closure system consists of butylrubber stoppers with fluororesin D Teflon film (grey) and aluminium crimp caps with polyproplyene disk (green).

Stability of the product

The submitted stability data sufficiently support the claimed shelf life of the drug product as described in the product information. The proposed shelf-life is 24 months when stored at 5 ± 3°C. Within the 24 month period, the product may be stored at room temperature (up to 30°C) for up to 6 months. A shelf life of 3 hours at ≤25°C is claimed after reconstitution.

The stability parameters are a subset of the drug product release parameters. The applicant presented real time/real temperature stability data with nine commercial lots (covering all dosage sizes) first stored at 5°C for 18 months followed by 6 months storage at 30°C/65% RH and 6 months storage at 25°C/60% RH. Further Baxter submitted stability data for the same lots at 5°C for 22 months followed by 2 months at 25°C/60%RH.

The container/closure system used for the stability investigations is the same as that intended for commercial use.

Drug product - water for injections (WfI)

WfI (5 mL size) is manufactured by Hameln Pharmaceuticals GmbH. The manufacture of WfI is carried out in accordance with GMP and is adequately documented. In summary, the manufacturing of sWfI starts with cleaning and sterilisation of the equipment, compounding, filtration and filling of the vials. The vials are then sterilised in an autoclave and 100% inspected. The final labelling and packaging is performed by Baxter.

WfI is in compliance with the requirements of the European Pharmacopeia, current edition. All quality control tests are performed by the manufacturer Hameln Pharmaceuticals according to Ph. Eur. current edition.

The container closure system complies with the relevant Ph. Eur. Monographs and consists of glass vial (colourless glass, type I Ph. Eur. 6 ml, DIN Standard 8362 part 1) and a stopper (chlorobutyl rubber stopper, Ph. Eur.).

The submitted stability data support for the 5 ml fill size the claimed shelf life of 2 years at +2-8°C, including partial storage at up to +30°C for not more than 6 months.

The basic stability studies were performed at the storage temperature of +5 ± 3°C, of +25 ± 2°C, and at +30 ± 2°C for 60 months, respectively and at +45 ± 2°C for 6 months. In addition, after storage at +5° ± 2°C for 54 months, samples were transferred to +25°C ± 2°C / 60% RH for additional 6 months. To expand the acceptable storage temperatures, two additional stability studies are currently in progress: one batch is currently investigated at +30° ±2°C for 48 months and further three batches at +30° ±2°C / 65% ± 5% RH for 60 months. All samples are stored inverted in the original containers material proposed for marketing.

For all batches stored at 5°C stability data comply with the specifications and demonstrate good stability profiles for all test parameters. The samples are stable over the test period (60 months) including a partial room temperature storage (up to +25°C) for a period up to 6 months.

Further information concerning the microbiological attributes, critical steps in the manufacture, analytical procedures used for quality control of WfI, and stability has been submitted as requested.

Baxject II

Baxject II has a needleless construction to avoid needle stick injuries. This device is currently used with licensed products. Both spikes (diluent and product side) are lubricated with silicone oil to reduce the penetration force while spiking through the rubber stopper. Baxject II device is intended for use with a single vial of Rixubis and sWfI only, therefore reconstituting and withdrawing a second vial into the syringe requires a second Baxject II device.

BAXJECT II device was determined to be suitable for use with Rixubis based on the following:

- rFIX potency by clotting for all devices (and samples) were within specification limit of 80 – 120%
- Total protein values for all devices (and samples) were within specification limit of 80 – 120%
- The aggregate and dimer values were for all devices (and samples) were within specification limits. In addition, no increase in aggregate and dimers were observed.
- No degradation bands were seen by SDS-PAGE and Western blot

The sub-visible particle counting by a very sensitive method (MFI) showed the particle count for ≥10 µm showed values above USP <788> for both the product and buffer even after electronic filter. However, visual imaging data showed that the particles were not protein, instead silicone (in both product and buffer). Therefore it is highly likely the protein particle complied with values within USP <788> limits.

Initially raised issues with the sub-visible particles have been discussed and solved.

Adventitious agents safety evaluation

The US and EU regulations require adequate safety margins for transmissible spongiform encephalopathy (TSE) agents in all medicinal products. The risk to rFIX with respect to TSE agents (i.e. prions) is minimized by the nature of the manufacturing process. In the pre-MCB stage of manufacturing process development FBS from countries with a controlled BSE risk was used. The MCB and WCB are stored in a serum-free medium. No animal- or human-derived materials are added during the manufacturing process or to the final container as excipients.

Cell banks were extensively controlled. The strategy of the cell bank testing for viral contamination, as proposed by the applicant, is deemed compliant with the ICH Guideline on the Quality of Biotechnological Products (CPMP/ICH/295/95). No viral particles other than retroviral-like particles normally seen in these cell types were observed.

The rFIX manufacturing process includes two dedicated virus inactivation/removal steps, SD treatment and 15nm nanofiltration, as well as a dedicated ion exchange chromatography step. Viral clearance studies were performed in small scale models. The design of these studies is deemed in compliance with CPMP/BWP/268/95 guideline. The results show that the two dedicated virus inactivation/removal steps and the ion exchange chromatography contribute efficiently to viral clearance. In addition, robustness of both steps has been demonstrated.

It is recommended as committed by the applicant to perform a virus clearance study with chromatography resin after the maximum cycle times (see 2.2.5).

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

The different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines. The information provided in the application demonstrates consistent batch-to-batch production of Rixubis achieving a well-defined quality for the drug substance and the drug product. The cell culture process, recovery and purification of the drug substance, nonacog gamma, are adequately controlled and validated. Appropriate drug substance specifications have been set. The drug substance has been adequately characterised using state-of the-art methods with regard to its physicochemical and biological characteristics. The manufacturing process of the drug product has been described and validated in sufficient detail. The quality of the drug product is controlled by adequate test methods and specifications. The data presented support the shelf-life proposed for Drug substance or Drug product. No excipients of human or animal origin are used in the product manufacture and there is no risk of contamination with viral or TSE agents by these ingredients.

In summary no major objections have been raised and most of the raised other concerns in Module 3 have been appropriately addressed in the responses to requests for information during the procedure. Nevertheless, recommendations addressing the characterisation of charged variants by IEF, the improvement of the recombinant FIX clotting assay, and the virus validation studies have been included.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

Overall, the data presented indicate that Rixubis is manufactured by a validated, controlled process taking into consideration relevant guidance documents. Batch release data confirm a product of consistent quality. The stability program is considered satisfactory. The results generated during the stability studies support the proposed shelf life and storage conditions as defined in the SmPC.

The dependency of clotting assay results on the aPTT reagent used in the one-stage clotting assay (some reagents giving results 40% above the labelled potency) has been noted. As requested, the Company follows the proposal made in the revised version of the core SmPC for FIX products (under public consultation) and includes high level information in the Rixubis SmPC that plasma factor IX activity results can be significantly affected by both the type of aPTT reagent and the reference standard used in the assay. This is also addressed in the RMP.

2.2.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends points for further investigation.

2.3. Non-clinical aspects

2.3.1. Introduction

The primary and secondary pharmacodynamics of nonacog gamma were investigated in a number of in vitro and in vivo studies. Nonclinical studies included primary pharmacodynamic, safety pharmacology, pharmacokinetic studies, single and repeat dose toxicity studies and one comparative immunogenicity study. Mouse models were used to test the pharmacodynamic effects of nonacog gamma; toxicology studies were performed in mice, cynomolgous monkeys, rats and rabbits by the iv route; pharmacokinetic studies were done in mice, rabbits and cynomolgous monkeys and safety pharmacology studies were carried out in rabbits and monkeys.

The recombinant FIX product BeneFIX and the plasma derived FIX product Mononine were used as comparators in the preclinical studies.

All pivotal nonclinical toxicity studies were conducted consistent with ICH Nonclinical Testing Guidelines and in compliance with the Good Laboratory Practice (GLP) Regulations.

2.3.2. Pharmacology

The pharmacological properties of nonacog gamma have been investigated in one in vitro study and in three in vivo FIX knock out mouse models (thromboelastography, tail tip bleeding, carotid occlusion).

Safety pharmacology aspects have been studied in 2 rabbit stasis models and in one Cynomolgus monkey study. PD drug interaction studies have not been performed.

Primary pharmacodynamic studies

The in vivo haemostatic effect was tested in the thromboelastography, tail tip bleeding and carotid occlusion mouse model of haemophilia B (F9 knock-out mice) compared to BeneFIX250 and Mononine.

Table 1: Primary pharmacodynamic studies

Pharmacodynamic Model	Species/ Strain	Method of Administration	Doses IU/kg	Gender and No. Per Group	Noteworthy Findings	GLP Compliance	Study Number
In vitro aPTT in plasma	Cynomolgus monkey, rat, human	n.a.	n.a.	n.a.	Dose-dependent shortening of aPTT-time	No	RD_VB_051002
Thrombelastography	FIX ko Mouse	Intravenous	10, 75	5M/5F	Dose-dependent efficacy shown. BAX326 efficacy similar to licensed rFIX.	No	WH0509
Tail Tip Bleeding	FIX ko Mouse	Intravenous	10, 25, 75, 100	8M/8F	Dose-dependent efficacy shown. BAX326 efficacy similar to FIX products.	No	WH0409
Carotid Occlusion Model	FIX ko Mouse	Intravenous	10, 75, 100	5M/5F	Dose-dependent efficacy shown. BAX326 efficacy similar to FIX products.	No	WH0309

These studies revealed a dose-dependent shortening of APTT-time (Activated Partial Prothrombin Time) in human, rat and Cynomolgus model systems demonstrating species cross-reactivity to rat and Cynomolgus with Rixubis; similar dose dependent efficacy and clot formation among Rixubis and the comparators; dose dependent efficacy in blood loss in the tail tip bleeding model.

The results of the in vitro assays such as the thrombin generation assay, as well as the efficiency of activation by FXIa and FVIIa in the presence of tissue factor and the capacity to bind to phospholipid vesicles sufficiently support primary PD of nonacog gamma as discussed under Quality aspects

Secondary pharmacodynamic studies

Secondary pharmacodynamic studies have not been submitted.

Safety pharmacology programme

Three in vivo safety pharmacology studies were conducted in rabbits and monkeys to assess the thrombogenic potential of nonacog gamma and its effects on the cardiovascular and respiratory system. The highest dose tested (750 IU/kg bw) in animals with normal haemostasis was 10 times the intended maximum human prophylactic clinical dose (75 IU/kg).

Table 2: Overview Safety Pharmacology Studies

Pharmacodynamic Model	Species/Strain	Method of Administration	Doses (IU/kg)	Gender and No. Per Group	Noteworthy Findings	GLP Compliance	Study Number
Thrombogenic Potential	Rabbit/ NZW	Intravenous	750	3M/3F	no thrombogenicity, Wessler scores lower than those of licensed rFIX and similar to licensed pdFIX	Yes	PV2420905
Thrombogenic Potential	Rabbit/ NZW	Intravenous	750	3M/3F	BAX326 showed Wessler scores lower comapred with those of licensed rFIX. After spiking FIXa into BAX326 in the amounts present in licensed rFIX, Wessler scores similar to licensed rFIX were obtained	No	AU0411W01
Cardiovascular Effects (Telemetry)	Cynomolgus Monkey	Intravenous	75, 450	4M	No adverse behavioral or clinical effects.	Yes	1933-013

Wessler score was used to measure thrombogenicity, this is based on 7-part ordinal scale (0, 0.5, 1, 2, 3, 3.5, 4), where 0 = no thrombus formation, 4 = high thrombus formation[1] (Wessler S., Reimer S. M., Steps M. C. (1959): Biologic assay of a thrombosis-inducing activity in human serum. J. Appl. Physiol. 14, 943-946). The Wessler scores obtained in the GLP conform study PV2420905 demonstrated that nonacog gamma was not thrombogenic in NZW rabbits after intravenous bolus application, with a comparable Wessler score for pdFIX Mononine, and was even slightly lower for rFIX-comparator BeneFIX after applying a 10-fold human clinical dose. FIXa analysis showed that a slightly higher Wessler score for the rFIX comparator BeneFIX is the consequence of a higher FIXa content (0.009 IU/ml for nonacog gamma and 0.106 IU/ml for BeneFIX). This was confirmed by FIXa spiking experiments with nonacog gamma in NZW rabbits (Wessler score 0.5 for BeneFIX, and 0.42 after addition of 0.1272 IU/mL FIXa). No adverse clinical effects and no effects on blood pressure, respiratory rate, body temperature, electrocardiogram or behaviour were observed in a GLP compliant telemetry study with cynomolgus monkeys.

Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies were conducted.

2.3.3. Pharmacokinetics

The following pharmacokinetic studies have been submitted:

Type of Study	Species Dose	Method & Frequency of Admin	Study No.	GLP
PK of BAX326, BeneFIX and Mononine	F9 KO mice	IV, Single dose	PV2360907/ GH2360907	Yes/ No
PK of BAX326, BeneFIX and Mononine	SD rat	IV, Single dose	PV2350905	Yes
PK of BAX326, BeneFIX and Mononine	Cynomolgus	IV, Single dose	1933-015	Yes

The methods used were the one-stage clotting assay (rat toxicokinetics PV2390901, Cynomolgus), a chromogenic assay (KO mouse) and an Ag ELISA (KO mouse, rat, Cynomolgus).

Absorption

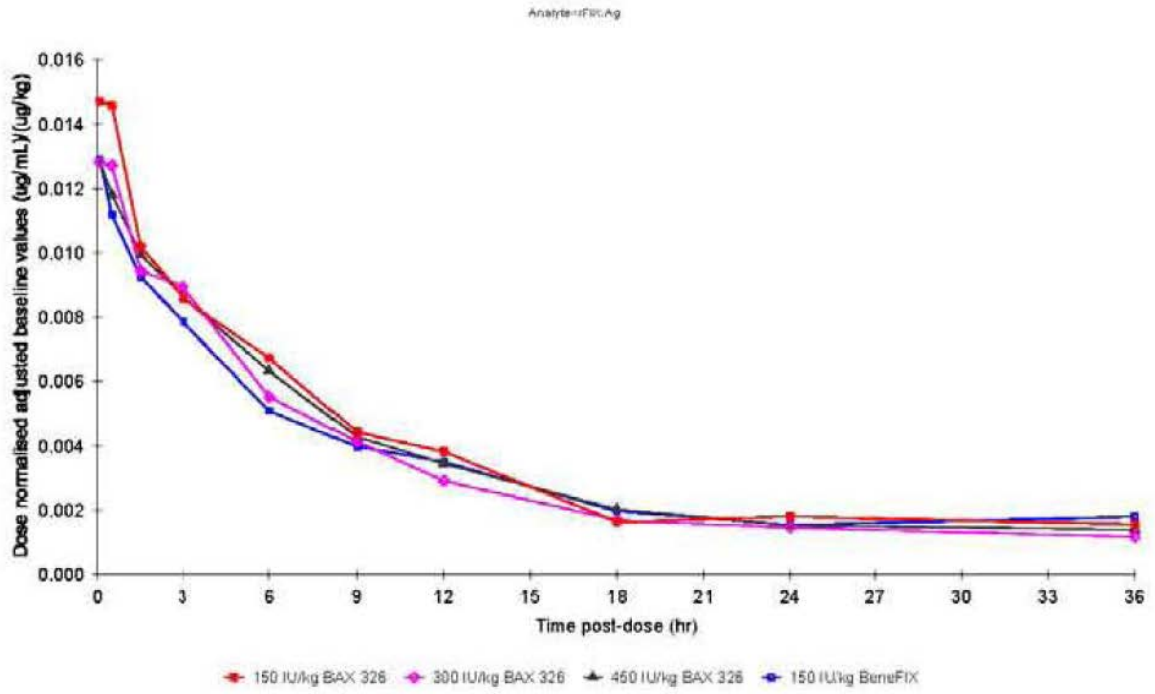
Absorption of nonacog gamma was evaluated following intravenous administration, which is the intended clinical route of administration. Pharmacokinetic assessments comprised FIX activity, maximum observed concentration (C_{max}), terminal half-life ($t_{1/2}$), area under concentration versus time curve ($AUC_{0-\infty}$), AUC from zero to the last measured time point ($AUC_{0-tlast}$), total body clearance (CL), volume of distribution at steady state (V_{ss}), in vivo recovery (IVR), incremental recovery (IR), mean residence time (MRT).

Single Dose (IV) in F9 knock-out mice - Study No.: PV2360907/ GH2360907
Design: Various doses (75, 200 and 750 IU/kg) of BAX326 were determined following IV administration in F9 KO mice and compared to that of 75 IU/kg doses of two marketed FIX products, BeneFIX and Mononine.
Outcome: PV2360907: similar $AUC_{(0-tlast)}$ FIX activity between BAX326, BeneFIX and Mononine, whereas Mononine showed a higher $AUC_{(0-tlast)}$ than both recombinant FIX products GH2360907: similar $AUC_{(0-tlast)}$ FIX antigen between BAX326 and Mononine, but 30% lower $AUC_{(0-tlast)}$ than BeneFIX; IVR, $t_{1/2}$, MRT similar to BeneFIX, but different to Mononine Disproportional increase in $AUC_{(0-tlast)}$, due to saturated metabolism at high doses

Single Dose (IV) in Sprague Dawley rats - Study No.: PV2350905
Design: Various doses (500, 1000 and 1500 IU/kg) of BAX326 were determined following IV administration in SD rats and compared to that of 500 IU/kg doses of two marketed FIX products, BeneFIX and Mononine.
Outcome: A biphasic decay could be observed for all items tested, Dose proportionality was observed (although the $AUC_{(0-tlast)}$ for the medium and high doses were slightly above the values expected statistically for dose proportionality, greater similarity of pharmacokinetic properties (MRT, $t_{1/2}$, IR, IVR, CL, V_{ss}) of BAX326 to the recombinant FIX product (BeneFIX) than to the plasma-derived FIX product (Mononine)

Single Dose (IV) in Cynomolgus - Study No: CLEM 1933-015
Design: Various doses (150, 300 and 450 IU/kg) of BAX326 were determined following IV administration in cynomolgus (2M/2F per group) and compared to that of 150 IU/kg doses of one marketed recombinant FIX product, BeneFIX.
Outcome: No clinical signs, no mortalities; bi-phasic decline in plasma concentrations of rFIX-antigen and rFIX-activity ($t_{1/2}$ 9 - 15 hours and 9 - 11 hours); Dose proportionality in the range of 150-450 IU/kg; $t_{1/2}$, CL, V_{ss} The apparent terminal elimination half-life, total body clearance and apparent volume of distribution of rFIX-antigen and rFIX-activity were dose-independent Conclusion: the pharmacokinetics of rFIX-antigen and rFIX-activity appeared to be consistent between BAX326 and BeneFIX

Figure 1: Combined Dose Normalised Mean Baseline Adjusted Plasma Concentrations (µg/mL) of rFIX-Antigen in Male and Female Cynomolgus Monkeys following single IV administration (150, 300 and 450 IU/kg nonacog gamma and 150 IU/kg BeneFIX



Distribution

No study using radiolabelled compounds or alternative methods were submitted.

Excretion

No excretion studies were submitted.

Pharmacokinetic drug interaction

No pharmacokinetic drug interaction studies were submitted.

Other pharmacokinetic studies

No other pharmacokinetic studies were submitted.

2.3.4. Toxicology

Table 3: Overview of the non-clinical toxicity studies

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (IU/kg) [a]	GLP Compliance	Study No.
Single-dose Toxicity	Mouse (C57BL/6NCrl)	Intravenous	Bolus	750, 4000, 7500	Yes	PV2380908
	Cynomolgus Monkey	Intravenous	Bolus	200, 750	Yes	1933-012
Repeat-dose Toxicity	Rat [Crl:CD(SD)]	Intravenous	14 doses every other day	200, 750	Yes	PV2390901

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (IU/kg) [a]	GLP Compliance	Study No.
	Cynomolgus Monkey	Intravenous	14 doses every other day	200, 750	Yes	1933-014
Genotoxicity	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Carcinogenicity	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Reproductive and Developmental Toxicity	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Local Tolerance	Rabbit (NZW)	Intravenous Intraarterial Paravenous	bolus	5 mL 5 mL 0.5 mL	Yes	PV2400901
Immunogenicity	Mouse Balb/c	Intravenous	bolus	2.5, 5, 10 µg/animal	Yes	PV2430911
Impurities	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Single dose toxicity

A single dose (IV) in mice (GLP Study No.: PV2380908) with the purpose to determine toxicity following a single IV administration at each test dose level, was performed using various doses (450, 700 and 4000 IU/kg) of Rixubis IV in C57BL/6NCrl mice (5M/5F per group) and compared to that of 750 IU/kg doses of BeneFIX, Mononine and control solutions.

No clinical signs of toxicity, no effects on body weight development over the 14 day recovery periodno effect on hematologic (hematocrit, platelet count) variables, no macro- or microscopic changes indicative of thrombogenicity or toxicity were noted. Rixubis was well tolerated and no signs for toxicity were observed in the single dose toxicity study in mice with doses up to 4000 IU/kg.

Escalating dose followed by a 28-day repeated dose IV (bolus) administration toxicity study in the cynomolgus monkey (GLP Study No.: 1933-012) with the purpose to determine whether a high dose with a several-fold safety margin over the anticipated clinical dose was tolerable and suitable for repeated application in the cynomolgus following IV (bolus) administrations was performed with doses of 200 and 750 IU/kg administered to two males and two females for the escalating dose phase and two males and two females for the repeated dose phase.

No treatment related changes were seen in the dose escalating dose phase.

A transient increase of APTT and fibrinogen in one male with detectable titers for neutralizing and binding antibodies was seen in the repeated dose phase; the NOAEL was observed at 750 IU/kg.

Repeat dose toxicity

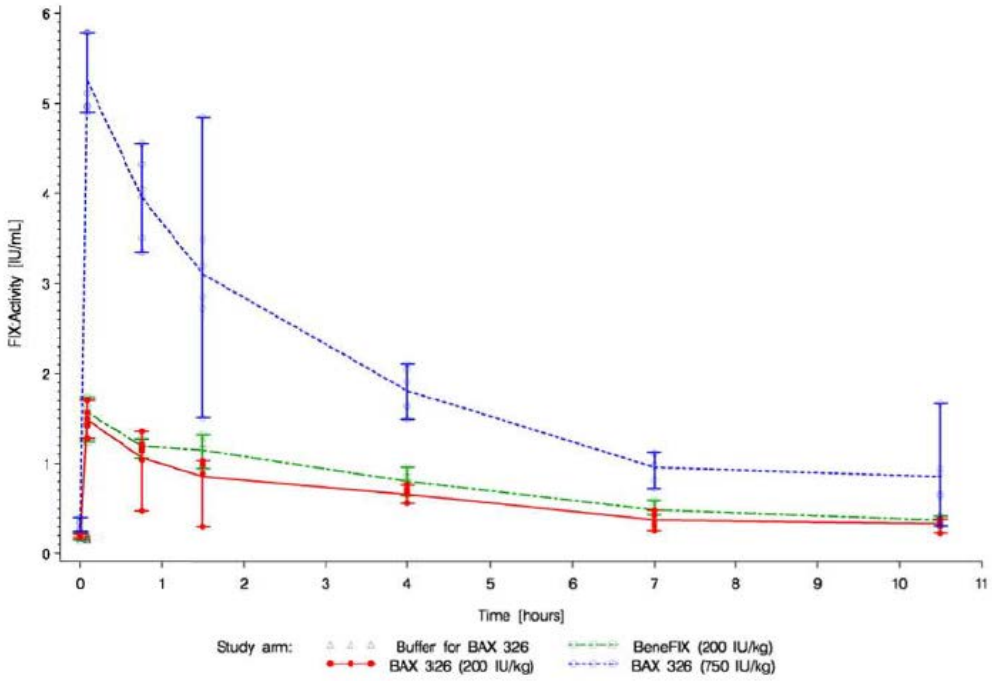
Two repeat dose toxicity studies were performed in rats and cynomolgus monkeys with daily IV dosing up to 750 IU/kg. The maximum treatment length was 28 days. These studies were performed in compliance with GLP.

Repeat dose (IV) in rats - Study No.: PV2390901	
GLP:	yes
Purpose:	Assessment of systemic toxicity in comparison with BeneFIX®, toxicokinetic analysis and anti-FIX antibody formation
Administration:	IV every other day

Duration:	28 days (14 applications) + 2 weeks recovery
Groups:	4 groups (10M/10F + 5M/5F as recovery animals) Group 1: 200 IU/kg BAX326 Group 2: 750 IU/kg BAX326 Group 3: 200 IU/kg BeneFIX Group 4: Control group
Toxicokinetics sampling:	0 (predose), 5, 45, and 90 minutes, 4, 7, and 10.5 hours after dosing
Outcome	no evidence of systemic toxicity; increase in APTT at 200 IU/kg BAX326 in females compared to predose values, but within the range in normal rats; increases of PT in the 750 IU/kg group of BAX326; no correlation with other clinical pathology or pathology findings; not consistent over time; PT-time at 200 IU/kg of BAX326 was similar to BeneFIX
Toxicokinetics: (see figure ..):	exposure throughout the whole study period similar AUC and C_{max} between BAX326 and BeneFIX
anti-FIX antibodies	no binding anti-human FIX antibodies after the end of the treatment period on day 28, Low titers of binding antihuman FIX antibodies after the recovery phase with 200 IU/kg BAX326 in 1/10 animals and after 750 IU/kg BAX326 in 2/10 animals. None of the antibodies had neutralizing activity

Repeated doses up to 750 IU/kg (14 applications) within 28 days were well tolerated in rats. No binding or neutralizing antibodies to FIX were detected on day 28 following dosing with either dose level of BAX326 or with 200 IU/kg of BeneFIX. However, positive anti-FIX binding antibody responses were detected in the recovery groups at day 42 (one rat dosed with 200 IU/kg and 2 rats dosed with 750 IU/kg BAX326), but none were detected in the BeneFIX group. A NOAEL was identified at 750 IU/kg.

Figure 2: Average concentration FIX activity on day 27



Repeat dose (IV) in cynomolgus monkeys - Study No.: 1933-014	
GLP:	yes
Purpose:	Assessment of systemic toxicity in comparison with BeneFIX, toxicokinetic analysis and anti-FIX antibody formation
Administration:	IV every other day
Duration:	28 days (14 applications) + 2 weeks recovery
Groups:	4 groups (5M/5F) - see table below
Toxicokinetics sampling	0 (predose), 5, 30, and 90 minutes, 3, 6, 9, 12, 18, 30, and 48 hours after dosing
Outcome	no evidence of systemic toxicity
Toxicokinetics: (see figure 2):	Dose proportional systemic exposure in all animals with up to 1.6-fold accumulation, $t_{1/2}$ ranged from 9 to 18 hours, $T_{1/2}$, Cl, V_{SS} dose- and time dependent, No gender related difference in the extent of systemic exposure of rFIX, consistent pharmacokinetics between BAX326 and BeneFIX
anti-FIX antibodies	binding anti-human FIX antibodies in 2M at 200 IU/kg BeneFIX, 2M and 2F at 200 IU/kg in BAX326 and 2M and 1F at 750 IU/kg BAX326; neutralizing Ab only in one M in the 200 IU/kg BeneFIX group on d28

Table 4: Treatment regime of the groups for repeated dose in cynomolgus monkeys

Group number	Group description	Dose level (IU/kg/day)	Application volume (mL/kg/day)	Animals/group		Necropsy after ...	
				Males	Females	4 weeks of dosing	2 weeks of recovery
1	Vehicle	0	2.1	5	5	3 M / 3F	2 M / 2 F
2	BeneFIX	200	0.6	5	5	3 M / 3F	2 M / 2 F
3	BAX 326	200	0.6	5	5	3 M / 3F	2 M / 2 F
4	BAX 326	750	2.1	5	5	3 M / 3F	2 M / 2 F

Figure 3: Plasma concentrations (IU/mL) of rFIX activity in Cynomolgus monkeys following IV administration of 200 and 750 IU/kg BAX326 and 200 IU/kg BeneFIX on day 1

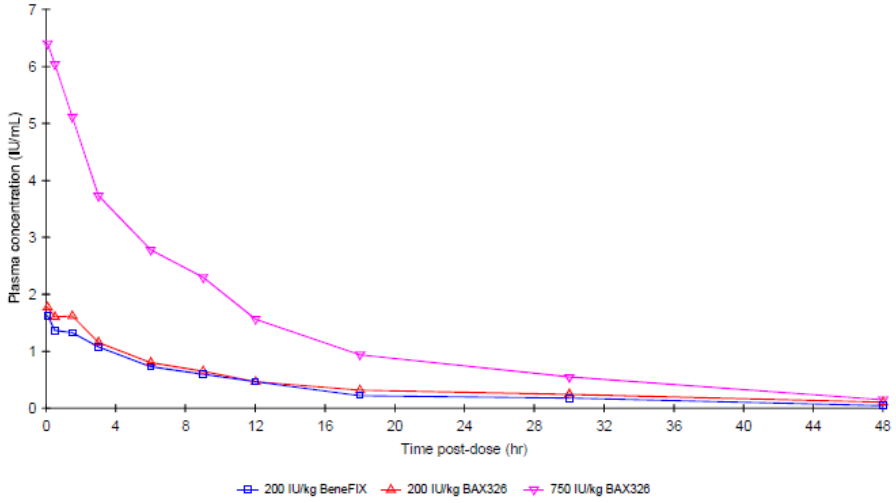
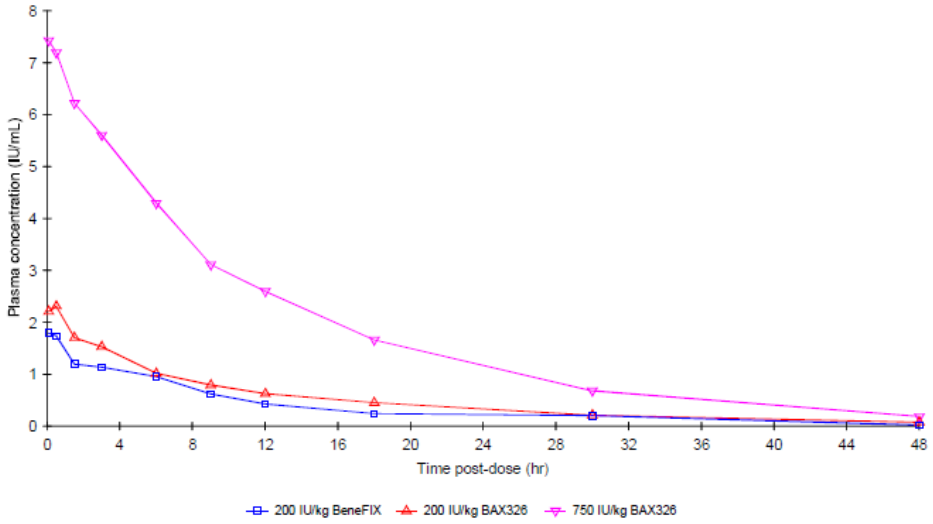


Figure 4: Plasma concentrations (IU/mL) of rFIX activity in Cynomolgus monkeys following IV administration of 200 and 750 IU/kg nonacog gamma and 200 IU/kg BeneFIX on day 27



Genotoxicity

No genotoxicity studies were submitted.

Carcinogenicity

No carcinogenicity studies were submitted.

Reproduction Toxicity

No reproductive and developmental toxicity studies, no dedicated fertility studies and no studies in juvenile animals were performed.

Fertility was assessed by histopathology of reproductive organs in the 28 days repeat dose toxicity study in cynomolgus monkeys.

Macroscopic and microscopic pathological investigations in repeat dose toxicity studies in rats (Study No. PV2390901) and cynomolgus monkeys (Study No. 1933-014) revealed no adverse effects on reproductive organs. Furthermore, no adverse effects have been reported on male fertility in clinical data of the licensed, recombinant FIX product BeneFIX.

Toxicokinetic data

Toxicokinetic data were obtained as part of the repeat-dose toxicity studies.

Local Tolerance

Table 5: Local tolerance study in the rabbit following intravenous, intraarterial and perivenous injection

Species/ Strain	Method of Administration	Doses (mg/kg)	Gender and No. Per Group	Noteworthy Findings	Study Number
Rabbit (NZW)	Intravenous Intraarterial Paravenous	5 mL 5 mL 0.5 mL	6M/6F	Clinical and histological findings after paravenous administration similar to the licensed, recombinant FIX product.	PV2400901

Rixubis was well tolerated after IV and IA administration. Paravenous administration of nonacog gamma (both tested lots) as well as BeneFIX led to local tissue reactions. The reactions were similar for the rFIX products with comparable potency.

Other toxicity studies

Comparative immunogenicity in Balb/c Mice - Study No.: PV2430911

The objective of this study was to assess the immunogenicity of BAX 326 in comparison to that of BeneFIX. In addition, development of antibodies against product impurities like host cell proteins were analyzed.

Table 6: Immunogenicity study after IV application in BALB/c Mice

Species/ Strain	Method of Administration	Duration of Dosing	Doses (µg/ i.v.dose)	Gender and No. Per Group	Noteworthy Findings	Study Number
Mouse (Balb/c)	Intravenous	8 doses given at weekly intervals	2.5, 5, 10	10M	No significant differences in the immunogenicity of BAX326 and the licensed, recombinant FIX product in the Balb/c mouse model	PV2430911

Anti-human FIX specific antibody development was observed after nonacog gamma treatment as well as after BeneFIX treatment. The highest anti-human FIX antibody titers were observed after BeneFIX treatment. No statistically significant differences in anti-human FIX antibody titres between treatment groups. No antibody development against potential impurities originating from the producing cell line (CHO protein) was observed.

Excipients

Excipients of Rixubis are sucrose, mannitol, sodium chloride, calcium chloride, L-histidine and polysorbate 80.

Four toxicity studies with Rixubis were conducted in mice, rats and cynomolgus monkeys with doses up to 7500 U/kg (see table 3) . According to the highest volume administered, formulation buffer with the excipients has been evaluated for adverse effects within these studies. Table 12 gives a summary about the toxicity studies with the highest exposure of animals with Rixubis excipients.

Table 7: Exposure of Mice, Rats and Monkeys in Toxicity Studies Compared with the Maximum Intended Human Clinical Dose of Rixubis

Excipient	Mouse Excipient Dose[1] (mg/m^2)	Rat Excipient Dose[2] (mg/m^2)	Monkey Excipient Dose[3] (mg/m^2)	Human Clinical Excipient Dose [4,5] (mg/m^2)
L-histidine	138.6	93	78.1	23.5
Calcium Chloride	19.7	13.2	11.1	3.34
Sodium Chloride	392.0	263.1	221.0	66.6
Mannitol	895.8	601.2	505.0	152.1
Sucrose	535.5	359.4	301.9	90.9
Tween 80	2.235	1.50	1.26	0.38

[1] The conversion factor to change from mg/kg to mg/m^2 is 3 for mice.
[2] The conversion factor to change from mg/kg to mg/m^2 is 6 for rats.
[3] The conversion factor to change from mg/kg to mg/m^2 is 12 for monkeys.
[4] Based on an intended human prophylactic dose of 75 IU/kg body weight with a potency of 400 IU/mL in the final drug product.
[5] Human body weight of 70 kg and a human body surface of 1.73 m^2 were assumed.

Based on table 7, the exposure to the excipients in the three studies was 5.9 (mice), 4.0 (rats), and 3.3 (monkeys) times higher than the maximal expected clinical exposure in humans.

Histidine

The acute toxicity of L-histidine (CAS 71-00-1) was reported to be low and in the grams/kg body weight range after i.v. administration to rodents. Animals received up to 138.6 mg/m^2 (mice) of histidine without any toxic effects.

Calcium Chloride

Calcium chloride (CAS 10043-52-4; dihydrate CAS 10035-04-8) is widely used excipients with a low acute toxicity. Animals received up to 19.7 mg/m^2 (mice) of calcium chloride without any toxic effects.

Sodium Chloride

Sodium chloride (CAS 7647-14-5) solutions are widely used for infusion with a concentration of 0.9% (9 g/L). Therefore, the exposure to 133.1 mg/m^2 is not considered to pose any risk. As expected, animals treated with up to 392.0 mg/m2 NaCl did not show any signs of toxicity.

Mannitol

Mannitol (CAS 69-65-8) is a sugar currently approved and marketed as Mannitol injection, USP for irrigation and diuresis. A typical dosage for use as a diuretic in humans is 50 mg/kg (2023 mg/m2) and for acute kidney failure is 100 - 200 g or 1.4 - 2.9 g/kg (57 - 117 g/m2).

In the single-dose toxicity study, mice were dosed with up to 895.8 mg/m2 mannitol in excipient controls with no effects attributable to mannitol.

The intended human exposure of 152.1 mg/m2 mannitol with Rixubis is more than 53 times below the typical diuretic parenteral starting dose of mannitol in humans of 8092 mg/m2 (200 mg/kg).

Sucrose

Sucrose ($C_{12}H_{22}O_{11}$; CAS 57-50-1) is a sugar that is recognized with GRAS status by the FDA. Sucrose is a substance of extremely low acute toxicity. Consumption of sucrose in large amounts or at frequent

intervals contributes to the development of dental caries, an adverse effect irrelevant for parenteral application.

The intended human exposure of 90.9 mg/m2 from the Rixubis dosing is not considered to pose any safety issue.

Tween 80

Tween 80 (Polysorbate 80, CAS 9005-65-6), a non-ionic surfactant widely used as an emulsifier, dispersant, or stabilizer in foods, pharmaceutical preparations, and cosmetics.

Tween 80 exposure due to Amiodarone treatment is 3182 times higher compared to that with Rixubis (see table 8). Tween 80 has been associated with drops in blood pressure in dogs (Intravenous Amiodarone and Tween 80) and has been associated with severe hypersensitivity reactions in some human patients using intravenous therapies with Tween 80 while appearing to be virtually non-toxic when ingested by humans.

Table 8: Tween 80 Concentrations in IV Pharmaceuticals and the Resulting Human Exposure

Drug	Tween 80 Concentration in Product [mg/mL]	Human Dose[1] [mL/24 h]	Human Tween 80 Exposure[2] [mg/day]	Factor to Tween 80 Exposure by BAX 326 Injection
Amiodarone (Antiarrhythmic drug)	100	21	2100	3182
Eraxis (Antifungal drug)	8.3	60	500	758
Neupogen (hrG-CSF)	0.04	23.3	0.93	1.4
Eprex (Erythropoiesis stimulating drug)	0.15	0.7	0.105	0.16
Aranesp (Erythropoiesis stimulating drug)	0.05	0.79	0.04	0.06
BAX 326	0.05	13.13	0.66	= 1

[1] Based on a clinical dose described in the package insert; for BAX 326 based on an intended human prophylactic dose of 75 IU/kg body weight.
[2] Based on an individual with 70 kg and 1.73 m^2 body surface.

The intended human clinical exposure in the BAX326 therapy is 0.38 mg/m2. This exposure is substantially less than in other currently marketed IV products and 23684 times lower than the Lowest Lethal Intravenous Dose (LDLo) in dogs of 9000 mg/m2.

2.3.5. Ecotoxicity / environmental risk assessment

The applicant did not submit an environmental risk assessment. They justified the lack of the report by stating that according to the European Medicines Agency Guideline *"Guideline on the environmental risk assessment of medicinal products for human use"*, vitamins, electrolytes, amino acids, peptides, proteins, carbohydrates and lipids are exempted from the requirement to provide an ERA as part of the Marketing Authorization Application because they are unlikely to result in significant risk to the environment. Therefore, the use, storage and disposal of nonacog gamma, a glycoprotein, does not pose a potential risk for the environment.

2.3.6. Discussion on non-clinical aspects

Nonacog gamma is a recombinant coagulation factor IX, it belongs to the pharmacotherapeutic group: antihaemorrhagics, blood coagulation factor IX (ATC code: B02BD04).

Pharmacology

One in vitro study and three in vivo studies characterized the haemostatic efficacy of Rixubis. The effects observed were comparable to other FIX products (rFIX Benefix, pdFIX Mononine).
Activated partial thromboplastin time (APTT) was assessed in Cynomolgus monkey, rat and human plasma. The in vivo haemostatic effect was tested in the thromboelastography, tail tip bleeding and carotid occlusion mouse model of haemophilia B (F9 knock-out mice) compared to BeneFIX250 and Mononine. The in vitro study aimed to verify the functional properties of nonacog gamma to interact with coagulation system of cynomolgus monkeys, rats and humans and by that verifying their suitability for the toxicological assessment of BAX326.

The in vivo studies were performed in three animal models using FIX ko mice to assess the efficacy of BAX326 in comparison with commercial recombinant FIX product, i.e. BeneFIX as well as with plasma derived product, i.e. Mononine. BAX326 was efficacious and a dose-dependent haemostatic effect was demonstrated with doses up to 75 IU/kg in the thromboelastography model and at 100 IU/kg in the tail-tip bleeding and the carotid occlusion model, respectively.

Safety pharmacology comprised investigation of thrombogenicity in rabbits and cardiovascular and respiratory endpoints including behavior and appearance in Cynomolgus monkeys. Rixubis was not thrombogenic at a dose of 750 IU/kg in a rabbit stasis model (Wessler-Test). Rixubis did not cause any adverse clinical, respiratory, or cardiovascular effects up to 450 IU/kg in cynomolgus monkeys. No further CNS endpoints were investigated but it was concluded that there is no evidence of functional or histological indices suggesting a possible toxicity on the CNS, based on clinical experience with marketed rFIX products and pharmacological studies investigating the impact of nonacog gamma on the cardiovascular and respiratory system.

Pharmacokinetics

Single dose pharmacokinetic studies showed systemic exposure in F9 knock-out mice, Sprague Dawley rats, and Cynomolgus monkeys.

Dose proportionality was observed in Cynomolgus monkeys, but not in rodents. Across all species, there was no gender-related difference in the extent of systemic exposure of rFIX antigen and rFIX activity and biphasic decay was observed for all items tested.

Toxicology

The toxicity studies submitted are considered appropriate for marketing authorization application of BAX326 as recombinant blood coagulation factor IX. Since the product is intended to be similar to BeneFIX, the approach to compare the toxicity profile of BAX326 with BeneFIX and with Mononine as plasma-derived FIX product is endorsed. Key elements of ICH S6 guideline pertaining to the selection of rodent and non-rodent species for acute toxicity and repeated dose toxicity have been followed.

Single application of up to 4000 IU/kg to mice revealed no treatment related findings. GLP conform repeat dose toxicity studies were performed in rats and monkeys with IV dosing every other day (28 days 14 applications). The limited duration of the studies is acceptable due to the species specific immunogenicity, although clinically chronic administration is intended. Administration of nonacog gamma up to 750 IU/kg was well tolerated. BeneFIX, (recombinant Factor IX marketed in the EU) was used as comparator.

In rats a slight increase in PT time in the 200 IU/kg Rixubis group in both sexes and an increase in APTT in the 200 IU/kg Rixubis group (in females only) was observed. Because of very low pre-dose values, the increased values are still within the range of normal APTT values and due to the absence of any pathological clinical signs, these findings are not considered to have a negative impact on the coagulation system.

Rixubis was well tolerated in single dose and repeated dose toxicity studies conducted in mice, rats and cynomolgus monkeys up to doses of 7500 IU/kg (single dose) and 750 IU/kg (repeated application). No signs of adverse effects related to Rixubis have been observed in the non-clinical setting. The NOAEL was identified at 750 IU/kg - the highest doses tested (rats and Cynomolgus), which is 10 times higher than the maximum intended prophylactic clinical dose of 75 IU/kg in humans.

In *Cynomolgus monkeys*, toxicokinetic analysis revealed that all animals in all dose groups were exposed to nonacog gamma; systemic exposure of rFIX-activity increased in an approximately dose-proportional manner across the dose range. The apparent terminal elimination half-life, total body clearance and apparent volume of distribution of rFIX-activity were dose- and time independent. There was no apparent gender-related difference in the extent of systemic exposure of rFIX-activity. The pharmacokinetics of rFIX-activity appeared to be consistent between nonacog gamma and the licensed, recombinant FIX product (nonacog alfa; BeneFIX).

An antigenicity study was performed in mice as well as one separate local tolerance study in rabbits.

In *rats*, no binding anti-human FIX antibodies were detectable after the end of the treatment period on day 28 in any of the treatment groups. Low titres of binding antihuman FIX antibodies were detected after the recovery phase of treatment with 200 IU/kg nonacog gamma in 1/10 animals and after 750 IU/kg nonacog gamma in 2/10 animals. No neutralizing antibodies have been detected.

In *Cynomolgus*, positive anti-FIX binding antibody responses were observed in two males receiving 200 IU/kg/day BeneFIX, two males and one female (200 IU/kg/day Rixubis) and two males and one female (750 IU/kg/day Rixubis). Neutralizing antibodies were confirmed for only one male monkey in the BeneFIX group.

No effects (macroscopically and histopathological) on the reproductive organs neither in male nor in female rats and cynomolgus were seen. Recombinant FIX has been widely used clinically and no adverse effects concerning fertility have been reported.

Based on results from the dose-escalation and repeat-dose toxicity studies in rats and cynomolgus, a no observed adverse effect level (NOAEL) was identified at 750 IU/kg - the highest doses tested. This NOAEL was 10 times higher than the maximum intended prophylactic clinical dose of 75 IU/kg in humans.

The immunogenicity of Rixubis was assessed in a comparative immunogenicity study against BeneFIX in Balb/c Mice (8 doses at weekly intervals). Additionally, development of antibodies against product impurities like host cell proteins were analysed. Immunogenicity of Rixubis was comparable to the recombinant FIX. Furthermore no development of antibodies against CHO proteins was observed.

Local tolerance of nonacog gamma was evaluated in a separate local tolerance study after IV, IA and paravenous injections in rabbits. Rixubis was well tolerated after IV and IA application at potencies of approximately 2000 and 3000 IU/vial. Paravenous application of Rixubis caused local tissue reactions comparable to BeneFIX.

There is no in vitro or in vivo evidence that rFIX has biologic properties other than those similar to endogenous FIX. For these reasons and in accordance with the ICH S6 guideline, no investigations on carcinogenicity, fertility impairment, and fetal development have been conducted. The omission of studies on genotoxicity, carcinogenicity and reproductive and developmental toxicity is justified, in line with ICH S6 (R1) guideline for preclinical safety evaluation of biotechnology-derived pharmaceuticals

which states that “the range and type of genotoxicity studies routinely conducted for pharmaceuticals are not applicable to biotechnology-derived pharmaceuticals and therefore are not needed.”
No reproductive testing has been conducted for Rixubis. According to the ICH Guideline S6 (R1) - preclinical safety evaluation of biotechnology-derived pharmaceuticals (EMA/CHMP/ICH/731268/1998) ‘The need for reproductive/developmental toxicity studies is dependent upon the product, clinical indication and intended patient population’. Haemophilia A is a hereditary sex linked disease, which generally only affects men. Plasma derived FIX and rFIX have been widely used clinically for many years with no signs of adverse effects concerning fertility. As such, separate reproductive and developmental toxicity studies for nonacog gamma are not expected to add any clinically relevant information.

Animal reproduction studies have not been conducted with factor IX. Adverse effects on fertility, postnatal development and reproduction as well as teratogenic effects are not expected in humans. Based on the rare occurrence of haemophilia B in women, experience regarding the use of factor IX during pregnancy and breast-feeding is not available. Therefore, factor IX should be used during pregnancy and breast-feeding only if clearly indicated (see section 4.6 of the SmPC). There is no information on the effects of rFIX on fertility.
Rixubis is contraindicated in patients with hypersensitivity to any of the excipients (listed in SmPC section 6.1.) From the non-clinical testing the excipients warrant no specific concerns, and exposure to Polysorbate 80 is substantially less than the Lowest Lethal Intravenous Dose (LDLo) in dogs and less than other currently marketed IV products however, due to its known potential for hypersensitivity reactions the risk is covered in the SmPC and PL by the above contra-indication.

The applicant has not performed an Environmental Risk Assessment (ERA) in accordance with the “Guideline on the Environmental Risk Assessment of the medicinal products for human use” (EMEA/CHMP/SWP/4447/00) – proteins are exempted because they are unlikely to result in significant risk to the environment.

2.3.7. Conclusion on the non-clinical aspects

Primary pharmacodynamic studies provided evidence that Rixubis demonstrated clear haemostatic efficacy. Several studies confirmed the comparability of the pharmacological and pharmacokinetic properties of Rixubis to commercially available FIX products. Regarding toxicology, no signs of adverse effects relating to Rixubis have been observed. The NOAEL was considered to be 750 IU/kg/day in rats and monkeys – the highest doses tested. No safety concerns in relation to human use have been identified.

The non-clinical programme submitted in support of the Marketing Authorisation application for Rixubis is sufficient. Relevant information and recommendations have been included in the SmPC sections 4.4, 4.6, 5.3.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Table9: Tabular overview of clinical studies

Study ID	Type of trial	Design	Number of dosed patients	Treatment	Duration	Primary Endpoint
250901	Phase 1/3 PK, safety and efficacy trial in PTPs ≥12	Part 1 PK: randomized, controlled, crossover with concurrent (active) control Part 2 prophylaxis or on demand treatment: open label, uncontrolled, Part 3: open label, uncontrolled repeat PK	73 subjects received BAX326; Of the 73 subjects, 28 were treated in Parts 1-3; 31 were treated in the prophylactic cohort and 14 in the on demand cohort of Part 2	Part 1: BAX326, commercial rFIX; infusion, IV, 75 ±5 IU/kg, single dose Part 2: prophylaxis: BAX326; infusion, IV, 50 IU/kg twice weekly for 6 months or ⩾50 EDs. OR on-demand: BAX3236; infusion, IV, 50 IU/kg as needed and 75 ± 5 IU/kg at each visit (to assess IR). Part 3: BAX326,; infusion, IV, 75 ±5 IU/kg, single dose	Prophylactic cohort: ~ 7-12 months On-demand cohort: 2-10 months; completed, final CSR available	AUC0-72 h/dose
251101	Phase 2/3 PK, safety and efficacy trial in PTPs <12	Prospective, open-label, uncontrolled, multicenter,	23 subjects: 11 subjects in <6-year age cohort; 12 subjects in 6-to-<12- year age cohort	PK: 75 ± 5 IU/kg for initial PK assessment and at each visit to assess IR Prophylaxis 50 IU/kg (ranging from 40-80 IU/kg) twice weekly with dose and frequency adjustments depending on the individual recovery and clinical response Treatment of acute bleeds At the investigator's discretion	6 months or at least 50 EDs; completed, final CSR available	All AEs possibly or probably related to BAX326
251002	Phase 3 Evaluate the safety and haemostatic efficacy of BAX 326 in the peri- and postoperative setting	Prospective, open-label, uncontrolled, multicenter	Approx. 30 subjects planned to receive BAX326 for perioperative haemostatic management; 14 subjects are included in the interim analysis comprising 11 major and 3 minor surgical interventions (iCSR)	Prior to surgery: Loading dose by intravenous infusion with BAX 326 sufficient to raise the level of FIX in plasma to 80-100% of normal for major surgeries and to 30-60% of normal for minor surgeries. After the loading dose(s), subjects are to continue to receive BAX326 as a bolus infusion depending on type of surgery, intensity and duration of the haemostatic challenge.	Dependent on the nature of the invasive procedure; ongoing, interim study report available.	intra- and post-operative haemostatic efficacy
251001	Phase 3 Further evaluate safety, immunogenicity, and haemostatic efficacy of BAX326 in subjects who completed Study 250901 or	Prospective, open-label, multicenter, uncontrolled, continuation study	100 subjects planned, all receiving BAX326	Either BAX326; infusion, IV Prophylaxis:50 IU/kg twice weekly for 6 months or ⩾50 EDs, OR modified prophylaxis determined by the investigator; OR on-demand treatment	Up to a maximum of 48 months; in the UK, ~12 months until subject has accumulated at least 150 EDs; ongoing:	AEs possibly or probably related to BAX326

	Paediatric Study 251101				no report available	

2.4.2. Pharmacokinetics

The PK characteristics of BAX326 were investigated in two clinical studies (see table 9):

- Study 250901: Comparison of the PK of BAX326 with the PK of a commercial rFIX (BeneFix) in PTPs >12 years of age
- Study 251101: PK of BAX326 in PTPs 0-12 years of age

In addition, in the surgery study 251002, IR was evaluated for all subjects and additional PK parameters only for subjects requiring major surgery.

Methods

Analytical methods

Validation of Bioanalytical and Immunogenicity Testing Procedures

Assay Type	Parameter Measured
FIX 1-stage clotting assay	FIX activity
FIX antigen ELISA	FIX antigen
FIX inhibitory antibodies	Inhibitory antibodies against FIX activity
Binding antibodies to FIX	Total Ig binding antibodies to FIX
Binding antibodies to Furin	Total Ig binding antibodies to Furin
Binding antibodies to CHO protein	Total Ig binding antibodies to CHO protein

FIX 1-stage clotting assay:

A validation of the factor IX one-stage clot activity assay was conducted in order to assess precision, accuracy, selectivity, stability and dilution capabilities of the assay to measure Baxter's recombinant FIX product (BAX326) when tested at three working dilutions (1: 10, 1:20 and 1 :40) in imidazole buffer. The assay measures FIX activity using a modified activated partial thromboplastin time (aPTT) based on an 8-point calibration curve consisting of normal pooled reference plasma diluted with imidazole buffer.

Validation was performed with recombinant FIX in the matrix of immuno-depleted FIX deficient plasma. Validation samples with 6 different concentrations, covering the expected range of the clinical study samples were prepared. For precision and accuracy, the validation samples were run in 6 independent assay runs. Based on all validation data it was concluded that the method is valid for bioanalytical analysis of FIX activity.

FIX Antigen ELISA

FIX antigen was analyzed with a commercially available ELISA test kit (Asserachrom IX:Ag, Diagnostica Stago, Asnieres, France). The assay is calibrated with a reference material, calibrated against the WHO standard. An 8-point reference curve is prepared and unknown samples are read from this curve. Validation was performed by spiking normal pooled human plasma into immuno-depleted FIX deficient plasma. Five different validation samples covering LLOQ up to a high concentration of FIX were prepared and measured in 6 different assay runs (precision and accuracy).

FIX inhibitory antibodies

Inhibitory (neutralising) antibodies to FIX are measured with a Nijmegen modification of the Bethesda assay. The validation was performed using commercially available FIX inhibitor plasma (Affinity Biologicals, Ancaster, Ontario, Canada). Three validation samples with titers at the LLOQ, mid and high concentration were prepared and analysed in 6 separate assay runs. Based on all validation data it was concluded that the method is valid for the determination of neutralising anti-FIX antibodies.

Binding antibodies to FIX, to Furin and to CHO protein was tested by relevant validated ELISA assays. All assays were validated with regards to inter- and intra-assay precision, specificity, linearity and robustness according to respective guidelines, Robustness was tested by analysing positive control samples that underwent five freeze/thaw cycles.

Absorption

N/A

Distribution

N/A

Elimination

N/A

Dose proportionality and time dependencies

N/A

Pharmacokinetics in target population

Pivotal Study 250901

This is a randomized, blinded, controlled, crossover PK Study of BAX326 and a commercial rFIX used as a comparator, administered as an IV infusion.

Subjects

Previously treated non-bleeding patients (with a minimum of 150 EDs) suffering from severe (FIX level <1%) or moderately severe (FIX level 1-2%) haemophilia B who were 12 to 65 years of age.

Design

Part 1 (PK part) was a randomized, blinded, controlled, crossover study to compare the PK parameters of BAX326 with BeneFIX in 27 subjects (26 evaluable). The data of the first 16 subjects who completed Part 1 were analysed for the presence of safety signals and reviewed by the independent DMC. In addition, the proposed dose to be used in Part 2 was confirmed by the DMC (per Protocol Amendment 2). Subjects were to be treated with a commercially available FIX concentrate other than BAX326 between the 2 infusions

in Part 1, as required. Thrombotic markers were also to be assessed at specified time points in Part 1. Part 3 was an open-label, uncontrolled repeat evaluation of the PK parameters of BAX326 after 26 ± 1 weeks of treatment in Part 2 in the 27 subjects who participated in Part 1. Subjects had to have a minimum of 30 EDs to BAX326 at the time of the PK assessment in Part 3. Thrombotic markers were also to be assessed at specified time points. Subjects who had not been exposed to BAX326 for a total of 50 days after completion of Part 3 were to continue receiving BAX326 until they had accumulated at least 50 EDs.

Treatment

In Part 1, subjects were to receive 2 infusions each, 1 infusion with BAX 326 and 1 infusion with BeneFIX, at a dose of 75 ± 5 IU/kg each in a randomized order.

The dose to be administered was calculated as 75 (IU) multiplied by patient body weight (kg). There was a minimum wash-out period of 5 days, preferably 7 days, prior to and between the PK infusions and the subject had to be in a non-bleeding stage. The PK evaluation had to be repeated if more than 28 calendar days elapsed between the 72 hour post-infusion time point of Infusion 1 and Infusion 2, or if the subject started bleeding during the 72 hour post-infusion time period.

Between Infusions 1 and 2, subjects were to be treated as needed with a commercially available FIX concentrate other than BAX 326 with a dose regimen as determined by the investigator.

All 27 subjects who participated in Part 1 were to enter Part 3 after 26 ± 1 weeks of treatment in Part 2 with a minimum of 30 EDs to BAX 326. Subjects participating in Part 3 were to be infused with a single dose of 75 ± 5 IU/kg of BAX 326. There had to be a minimum wash-out period of at least 5 days, preferably 7 days, between the last infusion in Part 2 of the study and the infusion for the repeat PK study, and the subject had to be in a non-bleeding stage. The PK evaluation could be repeated if the subject started bleeding during the 72 hour post-infusion time period

PK endpoints

Primary PK endpoint: To determine PK equivalence of BAX326 with a commercially available rFIX product used as a comparator, as measured by the area under the plasma concentration versus time curve from 0 to 72 hours post-infusion ($AUC_{0-72\ h}$ /dose) after a single dose of 75 ± 5 IU/kg

Secondary PK endpoints: $AUC_{0-\infty}$ /dose (area under the plasma concentration versus time curve from time 0 to infinity), MRT (mean residence time), CL (clearance), IR (incremental recovery), $T_{1/2}$ (elimination phase half-life), Vss (volume of distribution at steady state), and IR over time.

FIX activity was measured using a one-stage clotting assay.

The principal objective of Part 1 was to demonstrate PK equivalence of BAX 326 with BeneFIX as measured by the AUC0-72h/dose with a single dose of 75 ± 5 IU/kg.

The PK parameters to be assessed in Parts 1 and 3 were: AUC 0-72 h/dose (area under the plasma concentration versus time curve from 0 to 72 hours post-infusion), AUC0-∞ /dose (area under the plasma concentration versus time curve from time 0 to infinity), MRT (mean residence time), CL (clearance), IR, T1/2 (elimination phase half-life) and Vss (volume of distribution at steady state). In addition, IR at 30 minutes post-infusion of BAX326 was also to be assessed over time in Part 2.

Blood samples for the PK evaluation were collected at the following time-points: 15±5 min, 30±5 min, 1 hour ±5 min, 3 hours ±10 min, 6 hours ±15 min, 9 hours ±30 min, 24 hours ±2 hours, 36 hours ±2 hours, 48 hours ±2 hours, 60 hours ±2 hours and 72 hours ±2 hours.

Results

PK equivalence of BAX326 and BeneFIX has been established, as the 90% CI for AUC0-72h /dose (and also for AUC0-72 h) – in both the PKPPAS and the PKFAS – is contained completely within the margins of equivalence defined as 80% to 125%.

Table 10: Results on PK parameters

Pharmacokinetic Parameters for Part 1 and Part 3, Pharmacokinetic Full Analysis Set					
PK Parameter	Statistic	Part 1: BAX326	Part 1: BeneFIX	Part 3: BAX 326	Ratio in BAX326 (Part1/Part3)
	N	28	28	25	25
AUC_{0-72h}/Dose (IU*hr/dL : IU/kg)	Mean (Std)	14.25 (3.18)	13.45 (2.94)	15.57 (3.32)	0.92 (0.10)
	Median	14.25	13.42	15.98	0.93
	Min; Max	9.51; 21.57	8.57; 20.63	9.52; 21.13	0.72; 1.09
AUC_{0-inf}/Dose (IU*hr/dL : IU/kg)	Mean (Std)	16.08 (3.29)	15.32 (3.28)	17.50 (3.73)	0.93 (0.10)
	Median	15.99	15.18	17.51	0.93
	Min; Max	10.97; 23.48	9.99; 22.84	10.59; 24.21	0.67; 1.15
AUC_{0-72h} (IU*hr/dL)	Mean (Std)	1074.40 (251.68)	1039.08 (277.25)	1164.92 (250.33)	0.93 (0.14)
	Median	1085.05	1030.41	1213.32	0.93
	Min; Max	696.07; 1576.55	650.08; 1827.68	753.85; 1626.81	0.67; 1.27
AUC_{0-inf} (IU*hr/dL)	Mean (Std)	1212.96 (261.58)	1180.41 (295.15)	1308.99 (287.69)	0.94 (0.14)
	Median	1235.63	1146.33	1317.57	0.94
	Min; Max	825.51; 1749.44	747.72; 1945.28	838.24; 1863.77	0.63; 1.33
IR at C_{max} (IU/dL : IU/kg)	Mean (Std)	0.87 (0.21)	0.77 (0.20)	0.95 (0.24)	0.93 (0.11)
	Median	0.87	0.74	0.94	0.97
	Min; Max	0.53; 1.35	0.44; 1.27	0.52; 1.38	0.66; 1.12
C_{max} (IU/dL)	Mean (Std)	66.65 (16.51)	60.39 (18.10)	73.24 (18.97)	0.93 (0.15)
	Median	67.40	56.70	72.50	0.92
	Min; Max	41.70; 100.30	33.60; 109.80	38.50; 106.30	0.62; 1.23
Half-life (hr)	Mean (Std)	26.34 (9.18)	27.09 (9.01)	24.67 (6.99)	1.14 (0.38)
	Median	24.58	25.72	23.67	1.05
	Min; Max	15.83; 52.34	17.59; 64.29	15.50; 42.20	0.55; 2.21

MRT (hr)	Mean (Std)	30.56 (7.01)	31.68 (7.04)	29.38 (4.36)	1.06 (0.20)
	Median	28.47	30.22	29.02	1.00
	Min; Max	22.25; 47.78	23.10; 60.70	21.32; 37.52	0.74; 1.66
CL (dL/(kg*hr))	Mean (Std)	0.0648 (0.0136)	0.0682 (0.0147)	0.0599 (0.0140)	1.09 (0.13)
	Median	0.0625	0.0659	0.0571	1.07
	Min; Max	0.0426; 0.0912	0.0438; 0.1001	0.0413; 0.0945	0.87; 1.49
V_{ss} (dL/kg)	Mean (Std)	2.01 (0.74)	2.16 (0.66)	1.75 (0.45)	1.14 (0.21)
	Median	1.74	1.95	1.64	1.11
	Min; Max	1.10; 3.94	1.19; 3.92	1.12; 2.72	0.81; 1.61
Dose (IU/kg)	Mean (Std)	57.32 (3.90)	77.42 (15.29)	74.81 (3.29)	NA
	Median	74.43	75.26	75.60	NA
	Min; Max	71.27; 91.38	65.45; 153.92	64.48; 79.40	NA

Table 10
Incremental Recovery at 30 min for BAX326
(Study 250901: Full Analysis Set)

Parameter	Statistic	Part 1/ Part 2: ED1	Part 2: Week 5	Part 2: Week 13	Part 3/ Part 2: Week 26	Completion/ Termination[a]
IR at 30min [IU/dL : IU/kg]	N	73	71	68	55	23
	Mean (Std)	0.79 (0.20)	0.83 (0.21)	0.85 (0.25)	0.89 (0.21)	0.87 (0.20)
	Median	0.78	0.79	0.83	0.88	0.89
	Q25 ; Q75	0.7 ; 0.91	0.68 ; 0.96	0.655 ; 1.015	0.75 ; 1.04	0.73 ; 0.96
	Min ; Max	0.26 ; 1.35	0.46 ; 1.48	0.14 ; 1.47	0.52 ; 1.29	0.52 ; 1.32
Change in IR from Part 1/Part 2: ED1 [IU/dL : IU/kg]	N	NA	71	68	55	23
	Mean (Std)	NA	0.03 (0.15)	0.05 (0.20)	0.10 (0.15)	0.11 (0.15)
	Median	NA	0.03	0.075	0.06	0.12
	Q25 ; Q75	NA	-0.09 ; 0.11	-0.07 ; 0.17	0.02 ; 0.17	0 ; 0.21
	Min ; Max	NA	-0.26 ; 0.53	-0.7 ; 0.49	-0.18 ; 0.65	-0.15 ; 0.38

[a] Completion Visit after Part3/Part2: Week26, if available, or Termination Visit anytime during the study
[generated by 250901_csra_pk.sas]

Table 11: Pharmacokinetic parameters for evaluable subjects (per-protocol analysis) are presented in the table below.

Parameter	Rixubis Initial cross-over study (N=25)	Rixubis Repeat Evaluation (N=23)

Parameter	Rixubis Initial cross-over study (N=25)	Rixubis Repeat Evaluation (N=23)
AUC_{0-72h} (IU.hr/dL)[a] Mean±SD Median (range)	1067.81±238.42 1108.35 (696.07-1571.16)	1156.15±259.44 1170.26 (753.85-1626.81)
Incremental recovery at C_{max} (IU/dL:IU/kg)[b] Mean ±SD Median (range)	0.87±0.22 0.88 (0.53-1.35)	0.95±0.25 0.93 (0.52-1.38)
Half-life (hr) Mean±SD Median (range)	26.70±9.55 24.58 (15.83-52.34)	25.36±6.86 24.59 (16.24-42.20)
C_{max} (IU/dL) Mean±SD Median (range)	66.22±15.80 68.10 (41.70-100.30)	72.75±19.73 72.40 (38.50-106.30)
Mean residence time (hr) Mean±SD Median (range)	30.82±7.26 28.93 (22.25-47.78)	29.88±4.16 29.04 (21.32-37.52)
V_{ss}[c] (dL/kg) Mean±SD Median (range)	2.02±0.77 1.72 (1.10-3.94)	1.79±0.45 1.74 (1.12-2.72)
Clearance (dL/(kg.hr)) Mean±SD Median (range)	0.0644±0.0133 0.0622 (0.0426-0.0912)	0.0602±0.0146 0.0576 (0.0413-0.0945)

[a] Area under the plasma concentration-time curve from time 0-72 hours post-infusion.
[b] Calculated as (C_{max}-baseline factor IX) divided by the dose in IU/kg, where C_{max} is the maximal post-infusion factor IX measurement.
[c] Volume of distribution at steady state

Incremental recovery 30 minutes after infusion was determined for all subjects in the combined phase 1/3 study at exposure day 1, at their week 5, 13, and 26 visits, and at the time of study completion or termination, if it did not coincide with the week 26 visit. The data demonstrate that the incremental recovery is consistent over time (see table below).

	Exposure Day 1 (N=73)	Week 5 (N=71)	Week 13 (N=68)	Week 26 (N=55)	At study completion / termination[b] (N=23)
Incremental recovery 30 min after infusion (IU/dL: IU/kg)[a] Mean±SD Median (range)	0.79±0.20 0.78 (0.26-1.35)	0.83±0.21 0.79 (0.46-1.48)	0.85±0.25 0.83 (0.14-1.47)	0.89±0.12 0.88 (0.52-1.29)	0.87±0.20 0.89 (0.52-1.32)

[a] Calculated as (C_{30min}-baseline factor IX) divided by the dose in IU/kg, where C_{30min} is the factor IX measurement 30 minutes after infusion.
[b] If not coinciding with week 26 visit.

PK Parameters - BAX326 Pilot vs. Commercial Product

Additional analyses were prepared for the FAS comparing the PK parameters a) within subjects before and after switching CTM, and b) between subjects who received one versus the other type of CTM.

Twenty-seven (27) out of 28 subjects in Part 1 received pilot scale material and 23 out of 25 subjects in Part 3 received commercial scale material. The comparison was performed in the 22 subjects who received both pilot (Part 1) and commercial (Part 3) BAX326 product. The Part 1/Part 3-ratio of PK parameters shows that higher AUC, IR and Cmax values were observed with the commercial product. The

Part 1/Part 3-ratio of MRT, half-life and CL demonstrates very similar values for both pilot and commercial products.

IR over time determined at 30 min post-infusion was assessed separately for subjects who received BAX326 pilot product and subjects who received BAX326 commercial product. Mean and median IR values were comparable for pilot and commercial product.

Surgery Study 251002

PK assessments: IR at 15 ± 5 minutes following loading dose prior to surgery (for all subjects); AUC0-72 h/dose, total AUC/dose, MRT, CL, IR, T1/2, and Vss – only for subjects ≥ 12 years of age undergoing major elective surgery who did not undergo a PK assessment in the pivotal study 250901 and for newly entering subjects requiring major surgery.

The 7 subjects for whom pre-surgical PK parameters were calculated from PK assessments in the surgery study received a mean (STD) dose of 75.75 (± 2.52) IU/kg of BAX326 (range: 71.80-78.79 IU/kg). The mean (STD) values (+ range) of the individual PK parameters were:

• AUC0-72 h/dose (IU hr/dL : IU/kg): 19.77 (± 7.88) (range: 14.46-37.26)

• AUC0-inf/dose (IU hr/dL : IU/kg): 21.74 (± 8.94) (range: 16.62-41.73)

• MRT (hr): 25.58 (± 4.56) (range: 18.57-31.16)

• CL (dL/[kg hr]): 0.0503 (± 0.0124) (range: 0.0240-0.0602)

• IR at 30 min (IU/dL : IU/kg): 1.06 (± 0.35) (range: 0.71-1.73)

• Cmax (IU/dL): 82.06 (± 26.93) (range: 56.80-134.40)

• T1/2 (hr): 21.50 (± 4.98) (range: 14.85-28.61)

• Vss (dL/kg): 1.28 (± 0.39) (range: 0.69-1.87)

Special populations-

Impaired renal function

N/A

Impaired hepatic function

N/A

Gender

Only males were included into the clinical study programme.

Race

Race of patients included in the PK part of study 250901		
	PKPPAS, N=25 n (%)	PKFAS, N=28 n (%)
White	19 (76.0)	21 (75.0)
Black or African American	1 (4.0)	1 (3.6)
Japanese	1 (4.0)	2 (7.1)

Native Latin American	3 (12.0)	3 (10.7)
Mestizo	1 (4.0)	1 (3.6)
Arabic	0 (0.0)	0 (0.0)

Weight

N/A

Elderly

No subjects above 59 years of age were included into the trials.

Children

Paediatric Study 251101: A Phase 2/3, Prospective, Uncontrolled, Multicentre Study Evaluating Pharmacokinetics, Efficacy, Safety, and Immunogenicity in Previously Treated Paediatric Patients < 12 years With Severe (FIX level <1%) or Moderately Severe (FIX level 1-2%) Haemophilia B

In the open-label, uncontrolled paediatric Study 251101, subjects were to receive an initial infusion with BAX326 at a dose of 75 ± 5 IU/kg for PK assessment. All 23 subjects in the PKFAS/FAS received the initial PK infusion, at a mean dose of 75.50 (± 3.016) IU/kg (median: 75.25 IU/kg; range: 70.0-83.6 IU/kg). There were 2 cohorts based on the age of the subjects: <6 years (n=11) and 6 to <12 years (n=12). Subjects in the <6-year age cohort (n=11) received median and mean (± STD) doses of BAX326 of 75.25 IU/kg and 74.86 ± 2.581 IU/kg, respectively (range: 70.4-79.3 IU/kg). Subjects in the 6-to-<12-year age cohort (n=12) received median and mean (± STD) doses of 75.14 IU/kg and 76.10 (± 3.366) IU/kg, respectively (range: 70.0-83.6 IU/kg).

Within each cohort, subjects were randomized to one of 2 blood sampling sequences for the PK assessment to reduce the burden of frequent blood sampling on the individual subject. There were a total of 7 post-infusion time points over 72 hours for taking blood samples for the PK evaluation. These were to be divided between 2 subjects, with the result that there were 4 post-infusion time points per subject

PK endpoints (secondary endpoints): AUC0-∞ /dose, total AUC/dose, MRT, CL, IR, T1/2, Vss, IR over time.

Table A	**PK Parameters for BAX326, Subjects <6 years, n=11**				**PK Parameters for BAX326, Subjects 6-<12 years, n=12**			
Parameter	Mean	SD	Median	Range	Mean	SD	Median	Range
AUC0-inf [IU·hr/dL]	723.7	119.00	717.2	488; 947	886.0	133.66	863.7	730; 1138
MRT [hr]	30.62	3.266	30.08	26.2; 36.2	25.31	1.830	24.74	23.7; 30.3
CL [dL/(kg·hr)]	0.1058	0.01650	0.1050	0.081; 0.144	0.0874	0.01213	0.0863	0.069; 0.108
Half-life [hr]	27.67	2.658	27.28	24.0;	23.15	1.582	22.65	21.8;

				32.2				27.4
Vss [dL/kg]	3.225	0.5233	3.157	2.65; 4.42	2.209	0.3165	2.185	1.70; 2.70

Table B	**Incremental Recovery at 30 min for BAX326**				
Age group	Parameter	Baseline	Week 5	Week 13	Week26
<6 years	N	10	11	10	10
	mean	0.586	0.630	0.676	0.647
	SD	0.1320	0.1028	0.1211	0.1274
	median	0.591	0.595	0.655	0.610
	range	0.31; 0.75	0.49; 0.80	0.51; 0.84	0.51; 0.84
6-<12 years	N	12	12	11	11
	mean	0.731	0.726	0.733	0.795
	SD	0.1615	0.1291	0.1400	0.1445
	median	0.714	0.701	0.703	0.783
	range	0.51; 1.00	0.48; 0.92	0.54; 1.00	0.56; 1.01

All 23 male subjects underwent an initial pharmacokinetic evaluation of Rixubis in a non-bleeding state as part of the combined phase 2/3 paediatric study. Subjects were randomised to one of two blood sampling sequences to reduce the burden of frequent blood draws on the individual subjects. The mean (± SD) and median dose of Rixubis in the full analysis set (n=23) was 75.50 ± 3.016 and 75.25 IU/kg, respectively, with a range of 70.0 to 83.6 IU/kg. The pharmacokinetic parameters were calculated from factor IX activity measurements in blood samples obtained up to 72 hours following the infusion.

Table 12: Pharmacokinetic parameters for all subjects (full analysis set)

Parameter	**< 6years (N=11)**	**6 - < 12 years (N=12)**	**All (N=23)**
AUC_{inf} (IU.hr/dL)[a] Mean±SD Median (range)	723.7 ± 119.00 717.2 (488-947)	886.0 ± 133.66 863.7 (730-1138)	808.4 ± 149.14 802.9 (488-1138)
Half-life (hr) Mean±SD Median (range)	27.67 ± 2.66 27.28 (24.0-32.2)	23.15 ± 1.58 22.65 (21.8-27.4)	25.31 ± 3.13 24.48 (21.8-32.2)
Mean residence time (hr) Mean±SD Median (range)	30.62 ±3.27 30.08 (26.2-36.2)	25.31 ± 1.83 24.74 (23.7-30.3)	27.85 ± 3.73 26.77 (23.7-36.2)
V_{ss}[b] (dL/kg) Mean±SD Median (range)	3.22 ± 0.52 3.16 (2.65-4.42)	2.21 ± 0.32 2.185 (1.70-2.70)	2.7 ± 0.67 2.69 (1.70-4.42)
Clearance (dL/(kg.hr)) Mean±SD Median (range)	0.1058 ± 0.01650 0.1050 (0.081-0.144)	0.0874 ± 0.01213 0.0863 (0.069-0.108)	0.0962 ± 0.01689 0.0935 (0.069-0.144)

[a] Area under the plasma concentration-time curve from time 0 to infinity.
[b] Volume of distribution at steady state

Incremental recovery 30 minutes after infusion was determined for all subjects in the combined phase 2/3 study at the initial pharmacokinetic evaluation (exposure day 1), at week 5, 13, and 26 visits, and at the time of study completion or termination, if it did not coincide with the week 26 visit. The data demonstrate that the incremental recovery is consistent over time across all paediatric age groups. See tables below.

Incremental recovery for Rixubis 30 minutes after infusion, both paediatric age groups:

Incremental recovery 30 min after infusion	PK (ED 1) All (N=22)	Week 5 All (N=23)	Week 13 All (N=21)	Week 26 All (N=21)
(IU/dL: IU/kg)[a] Mean±SD Median (range)	0.67 ±0.16 0.69 (0.31 – 1.00)	0.68 ± 0.12 0.66 (0.48 – 0.92)	0.71 ± 0.13 0.66 (0.51-1.00)	0.72 ± 0.15 0.734 (0.51-1.01)

[a] Calculated as (C_{30min}-baseline factor IX) divided by the dose in IU/kg, where C_{30min} is the factor IX measurement 30 minutes after infusion.

Incremental recovery for Rixubis 30 minutes after infusion, paediatric patients < 6 years:

Incremental recovery 30 min after infusion	PK (ED 1) All (N=10)	Week 5 All (N=11)	Week 13 All (N=10)	Week 26 All (N=10)
(IU/dL: IU/kg)[a] Mean±SD Median (range)	0.59 ± 0.13 0.59 (0.31-0.75)	0.63 ± 0.10 0.6 (0.49-0.80)	0.68 ± 0.12 0.66 (0.51-0.84)	0.65 ± 0.13 0.61 (0.51-0.84)

[a] Calculated as (C_{30min}-baseline factor IX) divided by the dose in IU/kg, where C_{30min} is the factor IX measurement 30 minutes after infusion.

Incremental recovery for Rixubis 30 minutes after infusion, paediatric patients 6 to < 12 years:

Incremental recovery 30 min after infusion	PK (ED 1) All (N=12)	Week 5 All (N=12)	Week 13 All (N=11)	Week 26 All (N=11)
(IU/dL: IU/kg)[a] Mean±SD Median (range)	0.73 ± 0.16 0.71 (0.51-1.00)	0.73 ± 0.13 0.70 (0.48-0.92)	0.73 ± 0.14 0.70 (0.54 – 1.00)	0.8 ± 0.14 0.78 (0.56-1.01)

[a] Calculated as (C_{30min}-baseline factor IX) divided by the dose in IU/kg, where C_{30min} is the factor IX measurement 30 minutes after infusion.

Pharmacokinetic interaction studies

N/A

Pharmacokinetics using human biomaterials

N/A

2.4.3. Pharmacodynamics

Studies on clinical pharmacodynamics have not been submitted.

2.4.4. Discussion on clinical pharmacology

Pharmacokinetic parameters of Rixubis were investigated in trials 250901 and 251101. In trial 250901 PTPs ≥12 years of age were included and in trial 250101 children <12 years of age were included. Study 250901 was a randomised, blinded, controlled, crossover pharmacokinetic study of Rixubis and a comparator conducted in non-bleeding male subjects (≥15 years of age) as part of the combined phase 1/3 pivotal study. The subjects received either of the products as a single intravenous infusion. The

mean (± SD) and median dose of Rixubis in the per protocol analysis set (n=25) were 74.69±2.37 and 74.25 IU/kg, respectively, with a range of 71.27 to 79.38 IU/kg. The pharmacokinetic parameters were calculated from factor IX activitiy measurements in blood samples obtained up to 72 hours following each infusion.

The pharmacokinetic evaluation was repeated for Rixubis in an open-label, uncontrolled study with Rixubis in male subjects who participated in the initial PK crossover study and had received prophylaxis with Rixubis for 26±1 weeks (mean ± SD) and accumulated at least 30 exposure days (EDs) to Rixubis. The Rixubis dose range in the repeat pharmacokinetics study was 64.48 to 79.18 IU/kg (n=23).

PK endpoints as well as blood sampling time points were according to the applicable guideline (Guideline on Clinical Investigation of Recombinant and Human Plasma-Derived Factor IX Products, EMA/CHMP/BPWP/144552/2009). Furthermore, a sufficient number of patients was included into the PK parts of the clinical studies. In study 250901 PK equivalence of BAX 326 compared to BeneFix - a licensed recombinant FIX product - was shown as the 90%CI for AUC_{0-72h}/dose as well as for AUC_{0-72h} (1067.81±238.42 IU.hr/dL) were within the margins of 80% to 125% (in both the PK per protocol population and the PK full analysis set). All other PK parameters are comparable between BAX326 and BeneFix.

Incremental recovery 30 minutes after infusion was determined for all subjects in the combined phase 1/3 study at exposure day 1, at their week 5, 13, and 26 visits, and at the time of study completion or termination, if it did not coincide with the week 26 visit. The data demonstrate that the incremental recovery is consistent over time.
A comparative PK study in PTPs ≥12 years included three adolescent subjects aged 12, 13 and 15, thus a minimum of PK data in older paediatric patients comparing Rixubis with BeneFix is available.

All 23 male paediatric subjects in trial 250101 underwent an initial pharmacokinetic evaluation of Rixubis in a non-bleeding state as part of the combined phase 2/3 paediatric study. Subjects were randomised to one of two blood sampling sequences to reduce the burden of frequent blood draws on the individual subjects. The mean (± SD) and median dose of Rixubis in the full analysis set (n=23) was 75.50 ± 3.016 and 75.25 IU/kg, respectively, with a range of 70.0 to 83.6 IU/kg. The pharmacokinetic parameters were calculated from factor IX activity measurements in blood samples obtained up to 72 hours following the infusion.

Incremental recovery 30 minutes after infusion was determined for all subjects in the combined phase 2/3 study at the initial pharmacokinetic evaluation (exposure day 1), at week 5, 13, and 26 visits, and at the time of study completion or termination, if it did not coincide with the week 26 visit. The data demonstrate that the incremental recovery is consistent over time across all paediatric age groups.

In general, PK parameters of BAX326 seem to be comparable with published data of other FIX products. The guideline requirement to employ at least 3 different lots is fulfilled within trial 250901 including a comparative analysis for all PK parameters according to lot. During the course of clinical study 250901 patients were switched from receiving BAX326 pilot to BAX326 commercial product, however, the comparability of pilot clinical batches and commercial clinical batches was further investigated and satisfactorily demonstrated.

The pharmacodynamic effects of FIX are closely associated to its PK parameters, therefore it was not necessary to conduct separate studies on clinical pharmacodynamics.

The BWP noted the dependency of clotting assay results on the aPTT reagent used in the one-stage clotting assay (some reagents giving results 40% above the labelled potency). As requested, the Applicant follows the proposal made in the revised version of the core SmPC for FIX products (under public consultation) and includes high level information in the Rixubis SmPC that plasma factor IX activity results can be significantly affected by both the type of aPTT reagent and the reference standard used in the assay.

2.4.5. Conclusions on clinical pharmacology

The clinical pharmacology aspects of nonacog gamma are adequately addressed and fulfil the requirements to support marketing authorization of Rixubis. All available information has been reflected within the SmPC.

2.5. Clinical efficacy

The haemostatic efficacy of Rixubis was investigated in three open-label, uncontrolled, multicentre studies: (see table 9)

250901, efficacy in 73 PTPs ≥12, 14 patients included in the on-demand arm and 59 in the prophylactic arm with treatment of break-through bleeds.

251101, efficacy in 23 PTPs <12 (11<6y; 12≥6y), prophylactic setting with treatment of break-through bleeds.

251002, efficacy in the surgical setting, 11 major and 3 minor surgical interventions in 14 PTPs ≥12

2.5.1. Dose response studies

Dose-finding studies have not been performed.

2.5.2. Main studies

Study 250901

This was a prospective, multicenter phase 1/3 study in 3 parts investigating PK, efficacy, safety, and immunogenicity of BAX326 in PTPs ≥ 12 years with severe or moderately severe haemophilia B.

Methods

- **Part 1** was a randomized, blinded, controlled, crossover study to compare the PK parameters of a single dose BAX326 (75 ±5 IU/kg) with those of the same dose of a commercial recombinant FIX (rFIX= Benefix: 75 ±5 IU/kg) in 27 subjects (26 evaluable) and to determine PK equivalence. Thrombotic markers were also assessed at specified time points.

- **Part 2** was an open-label, uncontrolled study of the haemostatic efficacy, safety, immunogenicity and HR QoL of BAX326 over 6 months with twice weekly prophylactic infusions with BAX326 or at least 50 EDs to BAX326, whichever occurred last, in 60 subjects in order to have 50 evaluable subjects (prophylactic cohort).

- **Part 3** was an open-label, uncontrolled repeat PK study with BAX326 (single dose of 75 ±5 IU/kg) in the subjects who participated in Part 1 and had been treated for 26 ± 1 weeks in Part 2 having accumulated at least 30 EDs to BAX326. Thrombotic markers were also assessed at specified time points.

Study Participants

Main Inclusion criteria

- Diagnosis of severe (FIX level < 1%) or moderately severe (FIX level ≤ 2%) haemophilia B (based on the one stage activated partial thromboplastin time [APTT] assay), as tested at screening at the central laboratory
- 12 to 65 years of age at time of screening

- Previous treatment with plasma-derived and/or recombinant FIX concentrate(s) for a minimum of 150 EDs (based on the subject's medical records) for subjects ≥ 6 years of age; a minimum of 50 EDs for subjects < 6. If a subject did not have a verifiable, documented history of 150 EDs, s/he could be enrolled if the following 2 requirements were met:

There were 100-150 EDs to any FIX product (plasma-derived or recombinant FIX concentrate(s), cryoprecipitate, or fresh frozen plasma) that are not fully documented, and

patient had participated in the IMMUNINE Protocol 050901 and accumulated either at least 50 EDs to Immunine or a total of at least 150 EDs to a plasma-derived and/or recombinant FIX concentrate prior to enrollment.

- No evidence of a history of FIX inhibitors (based on the subject's medical records). If a verifiable, documented history was unavailable, the subject could be enrolled if s/he had participated in Study 050901 for at least 50 EDs to IMMUNINE prior to enrollment
- Immunocompetent as evidenced by a CD4 count ≥ 200 cells/mm3
- Human immunodeficiency virus (HIV) negative or HIV+ with a viral load <200 particles/μL ~ <400,000 copies/mL

Main Exclusion criteria

- History of allergic reaction, e.g. anaphylaxis, following exposure to FIX concentrate(s)
- Known hypersensitivity to hamster proteins or rFurin
- Evidence of an ongoing or recent thrombotic disease, fibrinolysis or disseminated intravascular coagulation (DIC)
- Abnormal renal function (serum creatinine >1.5 times the upper limit of normal)
- Active hepatic disease with alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels >5 times the upper limit of normal
- Severe chronic liver disease as evidenced by, but not limited to, any of the following: International Normalized Ratio (INR) > 1.4, hypoalbuminemia, portal vein hypertension including presence of otherwise unexplained splenomegaly and history of esophageal varices
- Platelet count <100,000/mL
- Subject is currently receiving, or is scheduled to receive during the course of the study, an immunomodulating drug (e.g. corticosteroid agents at a dose equivalent to hydrocortisone greater than 10 mg/day, or α-interferon) other than anti-retroviral chemotherapy
- History of FIX inhibitors with a titer ≥ 0.6 BU; Detectable FIX inhibitor at screening, with a titer ≥ 0.6 BU
- Weight is < 35 kg or > 120 kg
- Active hepatic disease with alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels > 5 times the upper limit of normal
- Diagnosis of an inherited or acquired haemostatic defect other than haemophilia B
- Clinically significant medical, psychiatric, or cognitive illness, or recreational drug/alcohol use that, in the opinion of the Investigator, would affect subject's safety or compliance

Treatments

The dose for prophylactic treatment was 50 IU/kg twice weekly, ranging from 40-60 IU/kg (up to 75 IU/kg permitted if necessary).
The following guidance was given for determining the dose for treatment of BEs. The FIX level was not to fall below the given plasma activity level in the corresponding period.

Degree of hemorrhage	FIX level required (%) (IU/dL)	Frequency of doses (hours)/Duration of therapy (days)
Early hemarthrosis, muscle bleeding or oral bleeding	20-40	Repeat every 24 hours. Duration: at least 1 day, until the BE as indicated by pain is resolved or healing is achieved.
More extensive hemarthrosis, muscle bleeding or hematoma	30-60	Repeat infusion every 24 hours for 3-4 days or more until pain and acute disability are resolved.
Life threatening hemorrhages	60-100	Repeat infusion every 8 to 24 hours until threat is resolved.

The required units were to be calculated according to the following formula: *Body weight (kg) x desired FIX rise (% or IU/dL) x {reciprocal of observed recovery}.*

Given an anticipated recovery of 0.8 [IU/dl]/[IU/kg], the required units were to be calculated using the following formula: *Body weight (kg) x desired FIX rise (% or IU/dL) x 1.3 IU/kg.*

Objectives

The objective of Part 1 of the study was to compare the PK parameters of BAX326 with those of BeneFIX and to determine PK equivalence (See Clinical Pharmacology section). Thrombotic markers were also to be determined at specified time points. Part 2 of the study was to assess the haemostatic efficacy, safety, and immunogenicity of BAX326 and HR QoL in those subjects receiving BAX326 for prevention and treatment of BEs as described in the following objectives:

- To monitor IR of BAX326 over time
- To evaluate the haemostatic efficacy of BAX326 in the management and prevention of acute BEs for a period of 6 months
- To evaluate safety in terms of BAX326-related AEs, as well as clinically significant changes in routine laboratory parameters (hematology/clinical chemistry) and vital signs
- To evaluate immunogenicity following a minimum of 50 EDs to BAX326
- To evaluate changes in health-related quality of life (HR QoL) and health resource use

The objective of Part 3 was to re-evaluate the PK parameters for BAX326 after a period of 6 months of treatment, in 27 subjects who had accumulated at least 30 EDs to BAX326, and to compare them with those determined in the same subjects who participated in Part 1. Thrombotic markers were also to be determined at specified time points.

Outcomes / endpoints

The efficacy endpoints consisted of primary and secondary PK endpoints (as discussed at the Pharmacokinetics section) and haemostatic efficacy endpoints. The following haemostatic efficacy endpoints were assessed during open-label prophylactic or on-demand treatment with BAX326:

- Treatment of bleeding episodes (BEs):
  - Number of infusions per BE
  - Overall haemostatic efficacy rating (excellent, good, fair, none) at resolution of bleed
  - Overall haemostatic efficacy ratings performed at 12 ± 1 and 24 ± 1 h time points as exploratory endpoints.

Table 13: Rating scale for treatment of bleeding episodes

Rating scale for treatment of bleeding episodes	
Excellent	Full relief of pain and cessation of objective signs of bleeding (e.g. swelling, tenderness, and decreased range of motion in the case of musculoskeletal haemorrhage) after a single infusion. No additional infusion is required for the control of bleeding. Administration of further infusions to maintain haemostasis would not affect this scoring.
Good	Definite pain relief and /or improvement in signs of bleeding after a single infusion. Possibly requires more than 1 infusion for complete resolution.
Fair	Probable and/or slight relief of pain and slight improvement in signs of bleeding after a single infusion. Required more than 1 infusion for complete resolution.
None	No improvement or condition worsens.

- Prophylaxis: ABR; Prophylaxis: Number of bleeding episodes beginning within 24 and 48 hours of an infusion as exploratory endpoint
- Consumption of BAX326:
  - Number of infusions and weight-adjusted consumption per month and per year.
  - Weight-adjusted consumption per event (for prophylaxis and on-demand).

The following safety endpoints were assessed throughout the study:

- Development of inhibitory and total binding antibodies to factor IX
- Development of antibodies to Chinese hamster ovary (CHO) proteins and rFurin
- Occurrence of severe allergic reactions, eg, anaphylaxis
- Occurrence of thrombotic events and clinically significant changes in thrombogenic markers during the PK parts of the study: prothrombin fragment 1.2 (F 1.2), thrombin-antithrombin III (TAT), D-dimer
- AEs related to Investigational Product (IP)
- Clinically significant changes in routine laboratory parameters (hematology and clinical chemistry), and vital signs.

In addition, the study endpoints included changes in the following health-related quality of life (HR QoL), health resource use (For subjects between 12 to 16 years of age and pharmacoeconomic parameters:

- Disease-specific: Haemo-QoL short version
- Generic: PedsQL™
- Health utility: EQ-5D
- General pain assessment through a visual analogue scale (VAS)

Health resource use; For subjects aged 17 years and older:

- Disease-specific: Haemo-A-QoL
- Generic: SF-36
- Health utility: EQ-5D
- General pain assessment through VAS
- Health resource use

Sample size

Sample Size for Part 1: PK Equivalence Test

The required sample size for PK equivalence test (part 1) was estimated to be 26 evaluable subjects. In order to allow for a minimum of 15% dropout rate, a total of 31 patients were randomized in Part 1. The sample size estimate was calculated for a Type-1 (α) error level of 0.05 and a Type-II (β) error level of 0.10 or 90% power, under the assumption that the true (population) means are equivalent. The within subject variability used (the square root of mean square error = 0.233) was estimated by increasing the variance observed in previous studies of Factor VIII by 10%.

Sample Size Considerations for Inhibitor Formation

A sample size of 54 evaluable subjects on prophylactic treatment was estimated for Part 2 to ensure that the upper limit of the 95% CI of the rate of subjects with an inhibitor was less than 10% if no or 1 subject developed inhibitors in the study.

In order to allow for a 10% dropout rate, a total of about 60 subjects were to participate in Part 2.

Sample Size Considerations for Treatment of Bleeding Episodes

An additional cohort of 15-20 subjects was to be enrolled to evaluate the haemostatic efficacy of BAX326 in the treatment of BEs in subjects receiving on-demand treatment only. This cohort was not intended to be used in comparison to the prophylaxis cohort, but to ensure adequate data on haemostatic efficacy of BAX326 in the treatment of BEs.

Randomisation

Eligible subjects were to be randomized to receive one of the following 2 PK infusion sequences: (1) BAX 326 followed by BeneFIX or (2) BeneFIX followed by BAX 326, with equal allocation in study Part 1. Using a centralized block randomization scheme, the number of subjects in each infusion sequence was balanced. Parts 2 and 3 of the study were non- randomized, open label.

Blinding (masking)

Subjects and investigators were only blinded in the crossover PK assessment in Part 1 of the study; Parts 2 and 3 of the study were non- randomized, open label.

Statistical methods

A Statistical Analysis plan was compiled. Three interim safety analyses were performed. The first interim safety review was performed by a DMC Safety Review Meeting, after 16 subjects had completed the PK evaluation of Part 1. The purpose was to ensure the safety of the IP and to confirm the proposed dose to be used in Part 2. A second interim safety review was performed after 11 subjects had completed Part 3 and had been evaluated for haemostatic efficacy, safety, and immunogenicity for a period of 50 EDs to enable the start of a clinical trial in children less than 12 years of age. The third interim safety review (full analysis) was introduced per Amendment 6; 50 subjects were to have completed Part 2 and had to be evaluated for haemostatic efficacy, safety and immunogenicity for a period of 50 EDs to BAX326 or 6 months, whichever occurred last.

Analysis Sets

The Full Analysis Set (FAS) was to comprise of all subjects who received at least 1 infusion during the study. The Pharmacokinetic Full Analysis Set (PKFAS) was to comprise all subjects who were randomized and received at least 1 infusion and who provided acceptable data for PK analysis.

The Pharmacokinetic Per Protocol Analysis Set (PKPPAS) was defined as a subset of the PKFAS, subjects who met the following additional criteria were to be included in the PKPPAS: as randomized and treated according to the randomization scheme, received both of the assigned infusions, and had no major violation affecting the PK period of the study. The analysis performed for PK parameters in Part 1 was to be presented for the PKFAS as well as for the PKPPAS. The analysis for the haemostatic efficacy endpoints, i.e., analysis of annualized bleed rate, analysis of treatment of BEs and analysis of consumption of BAX326, was to be performed on the FAS. Also, the analysis of safety endpoints was to be performed on the FAS.

Analysis of Annualized Bleed Rate

Annualized Bleed Rate (ABR) during prophylaxis and on-demand treatment in Part 2 was to be calculated as (Number of bleeding episodes/observed treatment period in days) * 365.25 and was to be compared to the ABR of a historical control. A meta-analytic approach was to be used to summarize the (annualized) bleed rates with on-demand infusions. The overall mean from the meta-analysis (μ1) assumed to be distributed N(μ1, SE1), was to be compared to the mean bleed rate under prophylactic regimen. The estimated mean μ2 was assumed to be distributed N(μ2, SE2). The null hypothesis μ1 = μ2 was to be tested against the one-sided alternative μ1 > μ2 using a z-test at one-sided 2.5% of significance level, for testing the bleeding reduction during a prophylactic regimen, using the formula:

$$Z = \frac{\hat{\mu}_1 - \hat{\mu}_2}{\sqrt{SE_1^2 + SE_2^2}}$$

Analysis of Treatment of Bleeding Episodes

The efficacy of bleeding treatment of BAX 326 was to be summarized. This included overall haemostatic efficacy rating at resolution of bleed, and the number of infusions and total weight-adjusted dose per bleeding episode, by anatomical site (joint/non-joint), cause (spontaneous/injury), severity (minor, moderate, major, and life/limb-threatening), and treatment regimen (prophylaxis and on-demand treatment). By Amendment 1 to the study protocol the assessment time point for the main overall haemostatic efficacy rating for the treatment of bleeding episodes was specified to be at resolution of

bleed. The overall haemostatic efficacy ratings performed at the 12±1 and 24±1 h time points were exploratory.

Safety Endpoints
Frequency counts and percentages were to be calculated for the following variables: occurrence of inhibitory and total binding antibodies to FIX, occurrence of antibodies to CHO proteins and rFurin, occurrence of severe allergic reactions, occurrence of thrombotic events and occurrence of clinically significant changes in thrombogenic markers.

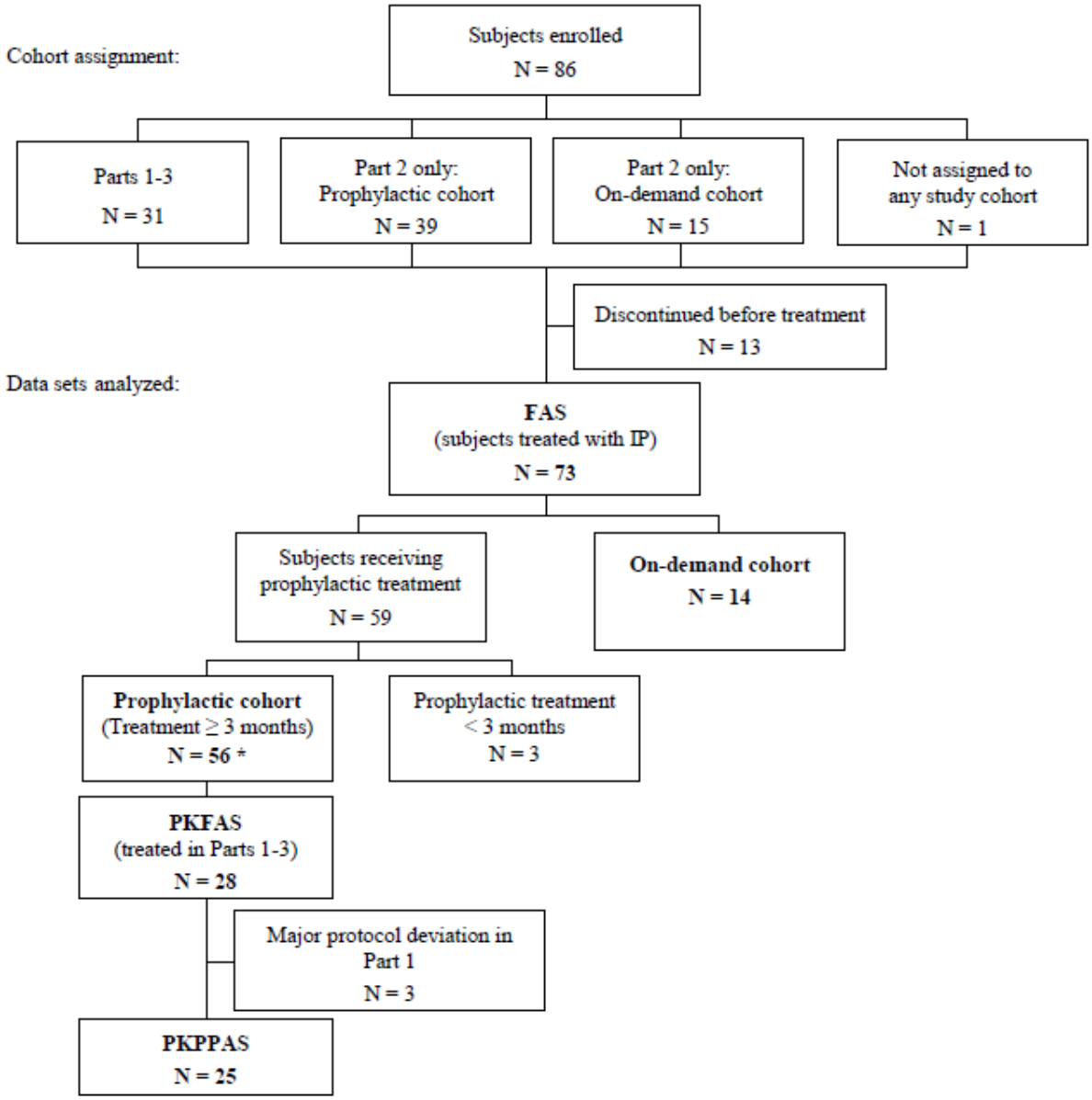
Missing Data
If a subject's weight was missing from any infusion record, the subject's last recorded weight was to be used to calculate the weight-adjusted dose.
Otherwise, no techniques were employed to adjust for missing data.

Results

Participant flow

A total of 73 subjects received IP during the study. They were further analysed by the type of treatment they received: Prophylactic treatment (n=59), Subjects with ≥3 months of prophylaxis (n=56) = prophylactic cohort, Subjects with ≥6 months of prophylaxis (n=29), on demand treatment (n=14).

Figure 10.1-1
Flow Chart for Study 250901



* Of 56 subjects who received prophylactic treatment for ≥ 3 months, 29 received prophylaxis for ≥ 6 months.

Recruitment

Clinical Study 250901 was conducted in Europe (Bulgaria, Czech Republic, Germany, Poland, Romania, Spain, Sweden, UK, Ukraine), Russia, South America (Argentina, Brazil, Chile, Colombia) and Japan. The sites with the highest enrolment were Site 1 (Bulgaria) and Site 18 (Ukraine) with 10 enrolled subjects each, followed by Site 26 (Russia) with 8 and Site 15 (Russia) with 6 enrolled subjects.

The trial was initiated on 29 July 2011 and completed on 3 May 2012 (total study duration 22 months).

Conduct of the study

There have been 7 amendments to the study protocol, with 3 global amendments and 4 local amendments specific to the US and/or Japan.

A total of 369 deviations were reported during the study. Of these, 37 were considered major deviations the majority of which concerned IP administration (e.g. wrong dose, 2 lots instead of one lot per infusion, 1000IU vials instead of 500IU vials in the PK part, infusion rate over 4mL/min).

Baseline data

All analyzed subjects were male.

The median age of all 73 subjects in the FAS was 33 years (range 12-59 years); there were three paediatric subjects aged 12, 13, and 15 years.

Most subjects were white (83.6%); the rest were Japanese (6.8%), Latin American/Mestizo (6.8%), Black or African American (1.4%) and Arabic (1.4%).

Thirty-nine (53.4%) subjects had a FIX activity level < 1%; while 34 (46.6%) subjects had a FIX activity level of 1-2%. FIX antigen levels were ≥ 1% in the majority of subjects (69.9%), with levels ≥ 40 % in 30.1% of subjects.

A missense mutation was diagnosed in 45.2% of subjects through genetic testing, followed by a nonsense mutation in 19.2%.

The majority of subjects (87.7%) had arthropathy at screening; 1-2 target joints were present in 41.1% of subjects; 12.3% of subjects had 3-4 target joints and a further 12.3% of subjects had > 4 target joints.

Only 13 (17.8%) of subjects had received prophylactic treatment prior to enrolment, whereas 27 (37%) had received on-demand treatment only and the remainder (33 subjects, 45.2%) both.

The most frequently reported previous FIX product used (in 15.1% of subjects) was the plasma-derived FIX product Immunine, manufactured and marketed by Baxter.

Numbers analysed

A total of 86 subjects have been enrolled in the study. Of these, 31 were enrolled for participation in Parts 1-3, 39 in the prophylactic cohort of Part 2 and 15 in the on-demand cohort of Part 2; one subject was not assigned to any study cohort. Of 73 subjects who received IP, 28 were treated in Parts 1-3, and 31 were treated in the prophylactic cohort and 14 in the on-demand cohort of Part 2. A total of 69 subjects completed the protocol (Parts 1-3: 26, prophylactic cohort: 29, on-demand cohort: 14). The following data sets were analyzed:

- Pharmacokinetic Per Protocol Analysis Set (PKPPAS) – n=25: comprises 25 subjects who participated in Parts 1-3 and completed Part 1 without any major protocol deviation
- PK Full Analysis Set (PKFAS) – n=28: comprises 28 subjects who participated in Parts 1-3 and completed Part 1; 3 subjects had a major protocol deviation in Part 1 (Subjects 090002, 310001 and 520001)
- Full Analysis Set (FAS) – n=73: comprise all 73 subjects who were exposed to IP. Within the FAS, subjects were further analyzed by the type of treatment they received in Part 2: Prophylactic cohort, n=56; On-demand cohort, n=14

Outcomes and estimation

<u>Annualized bleeding rate, ABR:</u>

The mean ABR in the prophylactic cohort (n=56) was 4.26 (± 5.80) (median: 1.99; range: 0-23.4). This was considerably lower than the mean and median historical on-demand bleed rates given by 53 of these subjects, which were 16.92 (± 16.72) and 13, respectively (range: 0-70). In 22/56 subjects who had no bleeds during the study, the subjects´ own historical on-demand bleed rate was 14.32 (± 14.96) (median: 10, range: 2-50) compared with a rate of 18.77 (± 17.87) (median: 15, range: 0-70) for 31/56 subjects with bleeds. The mean rate of joint bleeds in the prophylactic cohort (n=56) in this study was 2.85 (± 4.25) compared with 1.41 (± 2.87) non-joint bleeds. The rate of spontaneous bleeds (mean: 1.72 ± 3.26; median: 0.00; range: 0.0-15.6) was comparable to the rate of bleeds caused by injury (mean: 1.70 ± 2.80; median: 0.00; range: 0.0-10.7).

Among the 14 subjects in the on-demand cohort of the FAS, who all had bleeds, the mean ABR was 33.87 (± 17.37) and the median ABR 26.98 (range: 12.9-73.1). This was slightly higher than the mean and median of these subjects´ own historical on-demand bleed rates, which were 24.50 (± 13.65) and 17, respectively, with a range of 12- 56. The mean rate of joint bleeds in the on-demand cohort was 29.88 (± 16.05) in the study versus 3.99 (± 5.26) for non-joint bleeds. The rate of spontaneous bleeds was higher (mean: 19.85 ± 12.90) than the rate of bleeds caused by injury (mean: 10.58 ± 13.58).

Dose:

The median dose per prophylactic infusion was 50.49 IU/kg in all 59 subjects on prophylaxis, and was 50.475 IU/kg in the prophylactic cohort with at least 3 months of prophylactic treatment (n=56).

The median number of prophylactic infusions per week was 1.8 (range: 1.5- 1.92) in all 59 subjects on prophylaxis and 1.805 (range: 1.5-1.92) in the prophylactic cohort with a minimum of 3 months of prophylactic treatment (n=56). Fifty-six (76.7%) subjects had 50 or more EDs to BAX326 during the study. In the prophylactic cohort (n=56), the mean number of EDs was 56.3 (± 7.2) (median: 54).

Treatment of bleeding episodes:

Of a total of 249 BEs in the FAS, 115 BEs occurred during prophylactic treatment and 134 BEs occurred in the on-demand cohort. By bleeding site, 197 were joint bleeds (of which 107 were bleeds into a target joint and 90 non-target joint BEs) and 52 were non-joint bleeds. While 130 bleeds were spontaneous bleeds, 90 were caused by injury; 29 were of unknown cause.

Of the total of 249 BEs, the majority (153; 61.4%) were treated with one infusion. Fifty-eight (23.3%) BEs were treated with 2 infusions, and 38 (15.3%) BEs were treated with more than 3 infusions. Minor and moderate bleeds were mostly treated with one infusion only (78.9% and 56.4%, respectively). Seven of 15 major bleeds (46.7%) were treated with more than 3 infusions.

Haemostatic efficacy at resolution of bleed was rated ‘excellent’ in 41.0% and ‘good’ in 55.0% of all treated BEs (total of 96.0%). Two percent of bleed treatments were rated as ‘fair’, and none had a rating of ‘none’. For the remaining 2.0%, no efficacy ratings were provided.

The mean total dose per bleed was 83.83 ± 58.82 IU/kg (median: 62.29 IU/kg, range: 25.5-372.1 IU/kg. The highest mean dose (94.5 ± 64.3 IU/kg) was administered for non-joint bleeds. A mean dose of 81.0 (± 57.1) IU/kg (median: 56.5 IU/kg) was administered for joint bleeds. A higher mean dose was administered for bleeds caused by injury (91.2 ± 62.5 IU/kg; range: 25-350 IU/kg) than for spontaneous bleeds (75.9 ± 50.3 IU/kg; range: 26-243 IU/kg). Three bleeds in 3 subjects (260004, 520001, 560001) were treated with more than 300 IU/kg.

Consumption:

All 73 subjects included in the FAS, consumed a total of 12,413,790 IU or – in terms of weight-adjusted consumption – 177,980 IU/kg. In the group consisting of Part 1-3 subjects and subjects from the

prophylactic cohort in Part 2 (n=59), the total consumption was 11,609,436 IU or 166,208 IU/kg. In the on-demand cohort of Part 2 (n=14), the total consumption was 804,354 IU or 11,772 IU/kg.

Median consumption per month was 347.8 IU/kg (mean: 356.1 ±61.3 IU/kg) in the Part 1-3 subjects and prophylactic cohort (n=59) and 167.3 IU/kg (mean: 166.6 ±64.2 IU/kg) in the on-demand cohort of Part 2 (n=14). By contrast, all 73 subjects in the FAS had a median consumption of 333.6 IU/kg per month (mean: 319.7 ± 97.0 IU/kg).

The median number of infusions administered per month in the Part 1-3 subjects and prophylactic cohort (n=59) was 6.7 (mean: 6.9 ± 1.0) while it was only 2.7 (mean: 3.1 ± 1.2) in the on-demand cohort (n=14).

Ancillary analyses

ABR by Target Joint

Of 56 subjects in the prophylactic cohort with a minimum of 3 months of prophylactic treatment, 35 reported target joints at screening. The overall mean ABR was higher in the group with target joints (mean: 5.25 ± 6.40, median: 3.10, range: 0.0-23.4) than in the 21 subjects with no target joints (mean: 2.61 ± 4.27, median: 0.00, range: 0.0-16.6). The mean ABRs of joint bleeds in subjects with target joints (n=35) compared to those with no target joints (n=21) were 3.81 ± 4.87 (median: 2.05, range: 0.0-21.5) and 1.25 ± 2.26 (median: 0.00, range: 0.0-7.7), respectively. The mean ABRs of non-joint bleeds were 1.44 ± 2.82 (median: 0.00, range: 0.0-10.7) and 1.36 ± 3.01 (median: 0.00, range: 0.0-10.4), respectively. Spontaneous BEs occurred at a mean rate of 2.41 ± 3.01 (median: 0.00, range: 0.0-15.6) in subjects with target joints compared with 0.58 ± 1.63 (median: 0.00, range: 0.0-6.4) in subjects with no target joints. The rate of BEs caused by injury was also slightly higher (mean: 2.07 ± 3.18, median: 0.00, range: 0.0-10.7) in the subjects with target joints (n=35) than in the 21 subjects with no target joints (mean: 1.07 ± 1.94, median: 0.00, range: 0.0-6.6).

Quality of Life

Subjects on prophylaxis reported statistically significant improvements between baseline and follow-up at approximately 6 months in the Physical Component Score (p = 0.0189) and the Bodily Pain (p = 0.0146) and Role Physical (p = 0.0162) domains of the SF-36 in addition to the EQ-5D VAS Score (p = 0.005). All other measures of HR QoL did not show significant changes over the course of the study in the subjects on prophylaxis. No significant differences in HR QoL were reported by the on-demand patients between baseline and follow-up.

Summary of main study

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 14: Summary of efficacy for trial 250901

Title: A Phase 1/3 Prospective, controlled, Multicenter Study Evaluating Pharmcokinetics, Safety, and Immunogenicitiy in Previously Treated Patients With Severe (FIX level <1%) or Moderately Severe (FIX level ≤2%) Haemophilia B	
Study identifier	250901

Design	This 3-part study was a Phase 1/3, prospective, multicenter study in PTPs with severe or moderately severe haemophilia B: • Part 1 was a randomized, blinded, controlled, crossover study to compare the PK parameters of BAX326 with BeneFIX in 27 subjects (26 evaluable) and to determine PK equivalence. Thrombotic markers were also assessed at specified time points. • Part 2 was an open-label, uncontrolled study of the haemostatic efficacy, safety, immunogenicity and HR QoL of BAX326 over 6 months with twice weekly prophylactic infusions with BAX326 or at least 50 exposure days (EDs) to BAX326, whichever occurred last, in 60 subjects in order to have 54 evaluable subjects (prophylactic cohort) • An additional cohort of 15-20 subjects were to receive on demand treatment with BAX326 until the last subject of the prophylactic cohort completed the study (on-demand cohort) • Part 3 was an open-label, uncontrolled repeat PK study with BAX326 in the subjects who participated in Part 1 and had been treated for 26 ± 1 weeks in Part 2 having accumulated at least 30 EDs to BAX326. Thrombotic markers were also assessed at specified time points.	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	22 months not applicable not applicable
Hypothesis	Exploratory: For all efficacy outcome measures descriptive statistics were presented.	
Treatment groups	Prophylactic cohort	BAX326 prophylactic treatment for 26±weeks or at least 50 EDs, N=56 (59 ITT)
	On-demand cohort	BAX326 on-demand treatment until the last subject of the prophylactic cohort completed the study, N=14
Endpoints and definitions	Treatment of BEs #Inf	number of infusions per BE
	Treatment of BEs HEff	overall haemostatic efficacy rating at resolution of bleed
	Prophylaxis ABR	annualized bleeding rate
	Prophylaxis #BE24-48	Number of BEs beginning within 24 and 48 hours of an infusion as exploratory endpoint
	Consumption of BAX326 #Inf/month Cons	Number of infusions and weight-adjusted consumption per month and per year
	Consumption of BAX326 Cons/event	Weight-adjusted consumption per event (for prophylaxis and on-demand)

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat		
Descriptive statistics and estimate variability	Treatment group	Prophylactic cohort	On-demand cohort	All
	Number of subject	56	14	70
	#Inf 1 2 ≥3	 49 (42.6%) 30 (26.1) 36 (31.3%)	 104 (77.6%) 28 (20.9%) 2 (1.5%)	 153 (61.4%) 58 (23.3%) 38 (15.3%)

	HEff excellent good fair none not reported	 25.2% 69.6% 3.5% 0.0% 1.7%	 54.5% 42.5% 0.7% 0.0% 2.2%	 41.0% 55.0% 2.0% 0.0% 2.0%
	ABR mean median range	 4.26 1.99 0-23.4	 33.87 26.98 12.9-73.1	 NA
	#BE24-48 within 24 hrs within 24-48 hrs within 48-72 hrs within 72-96 hrs after 96 hrs	 21 (0.78%) 34 (1.26%) 36 (1.34%) 11 (0.41%) 20 (0.74)	 NA	 NA
	#Inf/month mean median range **Cons** mean median range	 6.9 6.7 3 - 10 356.1 347.8 159 - 516	 3.1 2.7 2 - 5 166.6 167.3 92 - 266	 6.1 6.6 2 - 10 319.7 333.6 92 – 516
	Cons/event (IU/kg) mean median range	 49.5 50.5 40 - 63	 93.2 87.1 34 - 210	 NA

Study 251101

This was a phase 2/3, prospective, uncontrolled, multicenter study evaluating pharmacokinetics, efficacy, safety, and immunogenicity in previously treated paediatric patients with severe (FIX level <1%) or moderately severe (FIX level 1-2%) Haemophilia B.

Methods

Study Participants

Inclusion criteria:

- Diagnosis of severe (FIX level <1%) or moderately severe (FIX level 1-2%) haemophilia B based on the one-stage aPTT assay, as determined by the central laboratory

- Subject age at time of screening: <12

- For subjects 6 to <12 years of age:

Previously treated with plasma-derived and/or recombinant FIX concentrate(s) for ≥150 EDs (based on the subject's medical records). If a subject did not have a verifiable, documented history of 150 EDs, s/he could be enrolled if the following critiera were met:

1) there were an estimated (not fully documented) 100-150 EDs to any FIX product (plasma-derived or recombinant FIX concentrate(s), PCC or FFP) (assumption based on the severity of disease and treatment history), and

2) the subject had participated in IMMUNINE Protocol 050901 and accumulated either ≥50 EDs to IMMUNINE or a total of ≥150 EDs to a plasma-derived and/or recombinant FIX concentrate prior to enrollment.

- For subjects **< 6 years of age:**

Previously treated with plasma-derived and/or recombinant FIX concentrate(s) for >50 EDs based on the subject' s medical records). If a subject did not have a verifiable, documented history of >50 EDs, s/he could be enrolled if the following criteria were met: a) there were approximately (not fully documented) 20-50 EDs to any FIX product (plasma-derived or recombinant FIX concentrate(s), PCC or FFP), and b) the subject had participated in IMMUNINE Study 050901 and accumulated ⩾30 EDs to IMMUNINE or a total of >50 EDs to a plasma derived and/or recombinant FIX concentrate prior to enrollment

- No history of FIX inhibitors (based on the subjects medical records).

If a verifiable, documented history was unavailable, the subject could be enrolled if s/he had participated in Study 050901 for ⩾30 EDs (< 6 years of age) or ⩾50 EDs (6 to <12 years of age) to IMMUNINE prior to enrollment.

- Willingness to have prophylactic treatment over a period of 6 months

- Immunocompetent as evidenced by a CD4 count ⩾ 200 cells/mm^3.

- Human immunodeficiency virus (HIV) negative or HIV+ with a viral load <200 particles/µL ~ <400,000 copies/mL

Exclusion criteria

History of FIX inhibitors with a titer ⩾0.6 Bethesda Units (BU) (as determined by the Nijmegen modification of the Bethesda assay or the assay employed in the respective local laboratory with the corresponding detection limit) at any time prior to screening

Detectable FIX inhibitor at screening, with a titer ⩾0.6 BU as determined by the Nijmegen modification of the Bethesda assay in the central laboratory

History of allergic reaction, e.g. anaphylaxis, following exposure to FIX concentrate(s)

Known hypersensitivity to hamster proteins or rFurin

Evidence of an ongoing or recent thrombotic disease, fibrinolysis or disseminated intravascular coagulation (DIC)

Abnormal renal function (serum creatinine >1.5 times the upper limit of normal)

International Normalized Ratio (INR) >1.4

Active hepatic disease with alanine aminotransferase (ALT) or aspartate

aminotransferase (AST) levels >5 times the upper limit of normal

Diagnosis of an inherited or acquired hemostatic defect other than hemophilia B

Platelet count <100,000/mL

Clinically significant medical, psychiatric, or cognitive illness, that in the opinion of the investigator, would affect subject's safety or compliance

Subject is currently receiving, or is scheduled to receive during the course of the study, an immunomodulating drug (e.g. corticosteroid agents at a dose equivalent to hydrocortisone greater than 10 mg/day, or α-interferon) other than antiretroviral chemotherapy

Treatments

The Rixubis dose for prophylactic treatment in paediatric patients in 251101 was 50 IU/kg twice weekly, ranging from 40 to 80 IU/kg, for a period of 6 months or for at least 50 EDs to BAX326, whichever occurred last The following guidance was to be employed, whenever possible, for determining the dose for treatment of bleeding episodes. The FIX level was not to fall below the given plasma activity level in the corresponding period.

Degree of hemorrhage	FIX level required (%) (IU/dL)	Frequency of doses (hours)/Duration of therapy (days)
Early hemarthrosis, muscle bleeding or oral bleeding	20-40	Repeat every 24 hours. Duration: at least 1 day, until the bleeding episode as indicated by pain is resolved or healing is achieved.
More extensive hemarthrosis, muscle bleeding or hematoma	30-60	Repeat infusion every 24 hours for 3-4 days or more until pain and acute disability are resolved.
Life threatening hemorrhages	60-100	Repeat infusion every 8 to 24 hours until threat is resolved.

The required units were to be calculated according to the following formula:

body weight (kg) x desired FIX rise (%) (IU/dL) x (reciprocal of observed recovery)

Based on an anticipated recovery of 0.7 [IU/dl]/[IU/kg], the required units were to be calculated using the following formula: *body weight (kg) x desired FIX rise (% or (IU/dL) x 1.4 IU/kg.*

Objectives

The primary objective of the paediatric study was to evaluate all AEs possibly or probably related to BAX326. The secondary objectives were the following:

- To evaluate the PK parameters of BAX326 in paediatric PTPs <12 years of age

- To monitor incremental recovery (IR) of BAX326 over time
- To evaluate the haemostatic efficacy of BAX326 in the management and prevention of acute bleeding episodes for a period of 6 months
- To evaluate safety in terms of immunogenicity for a minimum of 50 exposure days (EDs), the occurrence of thrombotic events, as well as clinically significant changes in routine laboratory parameters (hematology/clinical chemistry) and vital signs
- To evaluate changes in HR QoL and health resource use.

Outcomes/endpoints

The efficacy endpoints included the following PK endpoints: Total AUC/dose , MRT, CL, IR, T1/2, Vss, IR over time as discussed in the Pharmacokinetics section and the following haemostatic efficacy endpoints:

Treatment of bleeding episodes: (Number of infusions per bleeding episode/ Overall haemostatic efficacy rating at resolution of bleed

Prophylaxis: annualized bleeding rate / Prophylaxis: number of bleeding episodes beginning within 24 and 48 hours of an infusion as exploratory outcome measures

Consumption of BAX326: number of infusions and weight-adjusted consumption per month and per year; weight-adjusted consumption per event

The following HR QoL parameters and health resource use were also assessed and changes in these parameters were part of the secondary study endpoints (see details above):

- For subjects between 2 to 7 years of age:
  - Generic: PedsQL™ (Parent-proxy versions: age group 2-4 years and age group 5-7 years)
  - Health resource use (hospitalizations, emergency room visits, doctor office visits, etc.)
- For subjects between 8 to 11 years of age:
  - Disease-specific: Haemo-QoL, short version
  - Generic: PedsQL™ Child version
  - Health resource use (hospitalizations, emergency room visits, doctor office visits, etc.)

Sample size

Ten subjects aged 6 to < 12 years and 10 subjects aged < 6 years were required. To account for a potential drop out, a total of 24 subjects consisting of 12 subjects per age cohort were to be enrolled.

Randomisation

The study was not randomised.

Blinding (masking)

This was an open-label study.

Statistical methods

The Full Analysis Set (FAS) comprised all subjects who received at least 1 infusion during the study. The Pharmacokinetic Full Analysis Set (PKFAS) comprised all subjects who have at least 1 plasma FIX activity level available during post-infusion time points. A non-linear mixed effects model approach (population PK) was implemented to analyze PK data. Individual empirical Bayesian estimates of PK parameters from the population PK model were reported using descriptive statistics. IR during the prophylactic treatment phase was displayed graphically over time for each subject and was also summarized by visit.

For all outcome measures descriptive statistics were presented by age stratum. Point estimates (mean or median) and 95% CIs were computed.

The annualized rate of bleeding episodes during prophylaxis was calculated for subjects who had adequate treatment time for bleeding rate assessment. The number of bleeding episodes beginning within 24 and 48 hours of the prophylactic infusion were summarized as exploratory outcome measures. The efficacy of BAX326 in the treatment of bleeds was summarized including overall haemostatic efficacy rating at resolution of bleed, and number of infusions and total weight-adjusted dose per bleeding episode. Product consumption was summarized, including average number of infusions and average weight-adjusted consumption per month / year, and average weight-adjusted consumption per event (prophylactic infusion and treatment of bleeds).

Frequency counts and percentages were calculated for the following variables: occurrence of inhibitory and total binding antibodies to FIX, occurrence of antibodies to CHO proteins and rFurin, occurrence of severe allergic reactions (e.g. anaphylaxis) and occurrence of thrombotic events.

For the Peds-QL, the total score and two summary scores (psychosocial health and physical health summary) were calculated for each subject, along with the scores for each of the Ped-QL domains. For the Haemo-QoL, a total score was calculated for each subject. For all scores, paired t-tests were employed to evaluate mean change from baseline at the Week 26 ± 1 follow-up. The analysis of health resource use data was descriptive in nature.

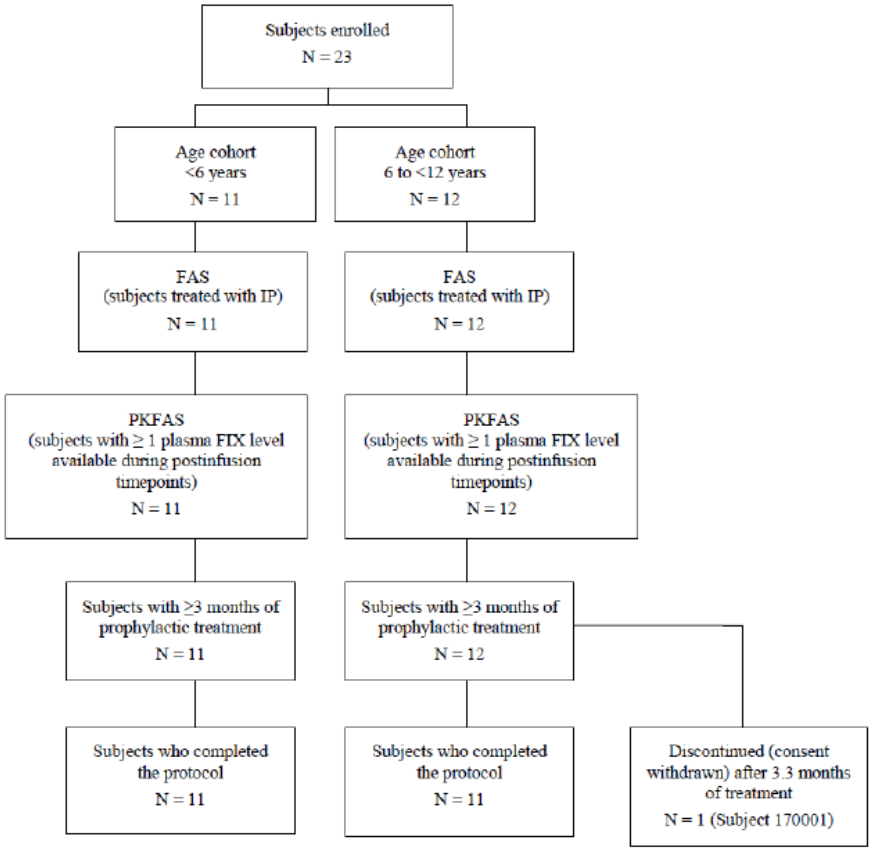
Results

Participant flow

A total of 23 patients were enrolled in the study (see figure X). Of these, 11 were <6 years and 12 were 6 - <12 years old. All 23 subjects were treated with IP and completed the PK assessment and were therefore included in the Full Analysis Set (FAS) and the Pharmacokinetic Full Analysis Set (PKFAS).

Twenty-two (22) subjects completed the protocol; one subject, who was 7 years old at the time of consent, discontinued the study (withdrawal by subject) after 35 EDs to BAX326 and a study duration of 4.34 months. Although the subject did not have ≥50 EDs to BAX326, he did complete >3 months (ie, 3.32 months) of prophylactic treatment.

Figure 10.1-1
Flowchart for Study 251101



Recruitment

Paediatric patients from 251101 study were enrolled at 11 study sites (3 sites in Russia with a total of 7 subjects, 3 sites in Poland with a total of 5 subjects; 2 sites in Romania with a total of 5 subjects, 1 site in Ukraine with 3 subjects, 1 site in the UK with 2 subjects and 1 site in India with 1 subject).

Conduct of the study

The paediatric study was conducted between December 2011 and May 2013 No protocol amendments were performed during this study. A total of 81 deviations were reported in 20 subjects during the study divided into 74 minor deviations related to the protocol schedule and seven major deviations (in 7 subjects) which mostly concerned BAX 326 administration.

Baseline data

Summaries of subject age at consent, weight, height and ABR prior to enrolment are provided below in Table

Table 15. Demographic and baseline characteristics- Study 251101

Age Group	Parameter	Statistic	PKFAS	FAS: Subjects with 3+ mon[a]	FAS: All
All Ages	Age at Consent [years]	N	23	23	23
		Mean (Std)	6.94 (3.394)	6.94 (3.394)	6.94 (3.394)
		Median	7.10	7.10	7.10
		Q25 ; Q75	3.35 ; 10.62	3.35 ; 10.62	3.35 ; 10.62
		Min ; Max	1.8 ; 11.8	1.8 ; 11.8	1.8 ; 11.8
	Weight [kg]	N	23	23	23
		Mean (Std)	23.8 (8.83)	23.8 (8.83)	23.8 (8.83)
		Median	21.2	21.2	21.2
		Q25 ; Q75	16.8 ; 33.0	16.8 ; 33.0	16.8 ; 33.0
		Min ; Max	11 ; 40	11 ; 40	11 ; 40
	Height [cm]	N	23	23	23
		Mean (Std)	118.6 (20.60)	118.6 (20.60)	118.6 (20.60)
		Median	122.0	122.0	122.0
		Q25 ; Q75	105.0 ; 136.0	105.0 ; 136.0	105.0 ; 136.0
		Min ; Max	74 ; 148	74 ; 148	74 ; 148
	ABR prior to enrollment	N	23	23	23
		Mean (Std)	6.8 (9.26)	6.8 (9.26)	6.8 (9.26)
		Median	2.0	2.0	2.0
		Q25 ; Q75	0.0 ; 13.0	0.0 ; 13.0	0.0 ; 13.0
		Min ; Max	0 ; 29	0 ; 29	0 ; 29

[a] Subjects received a minimum of 3 months of prophylactic treatment with BAX326
[generated by 251101_csra_gendemo.sas]

Summaries of gender, race, ethnicity, gene mutation, FIX activity, FIX antigen level, arthropathy at screening, number of target joints at screening and prior treatment (on-demand or prophylaxis) are provided below in Table

Table 16. Demographic and baseline characteristics-Categorical data – Study 251101

				FAS	
Age Group	**Parameter**	**Category**	**PKFAS n (%)**	**Subjects with 3+ mon[a] n (%)**	**All n (%)**
All Ages	Number of Subjects	N	23	23	23
	Gender	Male	23 (100.0)	23 (100.0)	23 (100.0)
		Female	0 (0.0)	0 (0.0)	0 (0.0)
	Age	<6 years	11 (47.8)	11 (47.8)	11 (47.8)
		6 - <12 years	12 (52.2)	12 (52.2)	12 (52.2)
	Race	White	22 (95.7)	22 (95.7)	22 (95.7)
		Indian	1 (4.3)	1 (4.3)	1 (4.3)
	Ethnicity	Not Reported	23 (100.0)	23 (100.0)	23 (100.0)
	Gene Mutation	Missense	11 (47.8)	11 (47.8)	11 (47.8)
		Nonsense	2 (8.7)	2 (8.7)	2 (8.7)
		Splice Site	1 (4.3)	1 (4.3)	1 (4.3)
		Deletion	2 (8.7)	2 (8.7)	2 (8.7)
		Frameshift	1 (4.3)	1 (4.3)	1 (4.3)
		Promoter	1 (4.3)	1 (4.3)	1 (4.3)
		Not Reported	5 (21.7)	5 (21.7)	5 (21.7)
	FIX Activity Level [%]	<1%	17 (73.9)	17 (73.9)	17 (73.9)
		1% - 2%	6 (26.1)	6 (26.1)	6 (26.1)
	FIX Antigen Level [%]	<1%	8 (34.8)	8 (34.8)	8 (34.8)
		≥1%	15 (65.2)	15 (65.2)	15 (65.2)
		1% - 2%	3 (13.0)	3 (13.0)	3 (13.0)
		>2% - 5%	0 (0.0)	0 (0.0)	0 (0.0)
		>5% - <40%	4 (17.4)	4 (17.4)	4 (17.4)
		≥40%	8 (34.8)	8 (34.8)	8 (34.8)
	Arthropathy at Screening	Yes	4 (17.4)	4 (17.4)	4 (17.4)
		No	19 (82.6)	19 (82.6)	19 (82.6)
	Number of Target Joints at Screening	0	18 (78.3)	18 (78.3)	18 (78.3)
		1 - 2	2 (8.7)	2 (8.7)	2 (8.7)
		3 - 4	2 (8.7)	2 (8.7)	2 (8.7)
		>4	1 (4.3)	1 (4.3)	1 (4.3)
	Prior Treatment	On-Demand	1 (4.3)	1 (4.3)	1 (4.3)
		Prophylaxis	16 (69.6)	16 (69.6)	16 (69.6)
		Both	6 (26.1)	6 (26.1)	6 (26.1)

[a] Subjects received a minimum of 3 months of prophylactic treatment with BAX326

All 23 enrolled subjects are included in the PKFAS and FAS and all 23 subjects completed ≥3 months of prophylactic treatment, therefore data for these populations are identical.

The mean subject age at the time of consent was 6.94 (± 3.394) years (median: 7.10 years, range:

1.8-11.8 years) (n=23). All subjects were male and all were white, except for one who was in the <6-year age cohort. The majority of subjects (17; 73.9%) had severe haemophilia B (FIX level <1%); 6 (26.1%) subjects had moderately severe haemophilia B (FIX level 1-2%). FIX antigen levels were <1% in 8 (34.8%) subjects and ≥1% in 15 (65.2%) subjects, with levels ≥40% in 8 (34.8%) subjects. Of the 18 subjects with available results of FIX gene mutation analysis, the majority (11 subjects; 47.8%) had a missense mutation. Two subjects (8.7%) had a nonsense mutation, another two subjects (8.7%) had a deletion, and one subject each had a splice-site (4.3%), a frameshift (4.3%) and a promoter mutation (4.3%).

Prior to treatment in the study, most subjects (16; 69.6%) had received prophylactic treatment, 1 subject had received on-demand treatment and 6 (26.1%) subjects had received both prophylactic and on-demand treatment.

The mean ABR prior to enrolment was 6.8 (± 9.26) (median: 2.0, range: 0.0-29). Only 4 subjects had arthropathy at screening; all of them were in the 6-to-<12-year age cohort and had received either prophylaxis only or prophylactic and on-demand treatment as prior treatment. Target joints were also only present in the 6-to-<12-year age cohort: 2 subjects had 1-2 target joints, 2 subjects had 3-4 target joints and 1 subject had >4 (ie, 5) target joints.

An overview of the medical history by system organ class (SOC) was presented. All 23 (100%) subjects had a history in the hematopoietic/lymphatic SOC due to the disease under investigation in this study. A high number of subjects had a medical history in the gastrointestinal SOC (18 subjects [78.3%], 9 in each age cohort). However, in 14 subjects this was solely due to vaccinations against hepatitis A and/or B. One subject was positive for both hepatitis B core and surface antibodies (mild severity) and another subject was positive for hepatitis B surface antibody (mild). A medical history in the musculoskeletal SOC was only documented for 5 (21.7%) subjects.

Four subjects who were all in the 6-to-<12-year age cohort, had a history of arthropathy; one subject, in the younger age cohort, had a history of hematomas of the occipital area and of the lower limbs in the musculoskeletal SOC. In 3 of 5 subjects with a history in the cardiovascular SOC this was due to the implantation of central venous catheters; additionally a case of vaccination against hepatitis B was recorded under the cardiovascular SOC and a case of a systolic murmur.

Treatment compliance

For PK assessment, 22 of 23 subjects in the PKFAS received the dose as prescribed (75 ± 5 IU/kg). One subject received a slightly higher dose (83.60 IU/kg), which was recorded as a major protocol deviation, however, the subject´s values were not excluded from the analysis.

For the prophylactic regimen (recommended dose of 50 IU/kg BAX326 twice weekly ranging from 40-80 IU/kg), the mean compliance in dose was 97.45 (± 8.848)% (median: 100%; range: 62.0-100%), and mean compliance in treatment frequency was 90.83(± 7.216)% (median: 92.45%; range: 74.5-100%). Compliance in dose and frequency was comparable between the two age cohorts.

Numbers analysed

The FAS comprised all 23 subjects who were exposed to IP. Within the FAS, subjects were analyzed by age cohort: <6 years (n=11) and 6 to <12 years (n=12). The Pharmacokinetic Full Analysis Set (PKFAS) included 23 subjects.

Outcomes and estimation

Overall annualized bleeding rate:

The mean ABR for all 23 subjects with ≥3 months of prophylactic treatment was 2.7 (± 3.14) (median: 2.0; range: 0.0-10.8). The mean ABR was slightly higher in subjects 6 to <12 years of age (n=12) than in subjects <6 years of age (n=11): mean ABR of 3.4 (± 3.93) (range: 0.0-10.8) in the older age cohort compared with 1.9 (± 1.89) (range: 0.0-5.4) in the younger age cohort. However, the median ABRs were comparable: median ABR of 2.0 in subjects <6 years of age compared with median ABR of 1.8 in subjects 6 to <12 years of age.

ABR by bleeding cause

The cause of BEs was mostly due to injury. In subjects of all ages, the mean ABR of BEs caused by injury was 2.4 (± 2.92) (median: 1.9; range: 0.0-10.8) compared with a mean ABR of spontaneous BEs of 0.2 (±0.66) (median: 0; range: 0.0-2.0). The ABR by cause (ie, spontaneous or injury) was evenly distributed among subjects of both age cohorts.

ABRs by Bleeding Site

In subjects of all ages (n=23), the mean ABR of joint Bes (major joints: wrist, elbow, shoulder, hip, knee, ankle) was 0.8 (± 1.76) (median: 0; range: 0.0-7.2). Given the presence of arthropathic joints and target joints in 4 and 5 subjects, respectively, in the 6-to-<12-year cohort, the mean ABR of joint BEs was higher in the older than in the younger age cohort where no arthropathy nor target joints were recorded at screening (see below Table 10). Of note, the ABR on non-joint BEs (soft tissue, muscle, body cavity, intracranial and other) was higher than the ABR of joint BEs. Indeed, in subjects of all ages, the mean ABR of non-joint BEs was 1.9 (± 2.42) (median: 1.9; range: 0.0-8.2) compared with a mean ABR of 0.8 (± 1.76) (median: 0; range: 0.0-7.2) for joint Bes.

ABRs by Bleeding Site, Cause and Baseline Characteristics

In the 12 subjects in the 6-to-<12-year age cohort, the ABR of joint BEs was higher in the 8 subjects who did not have arthropathy at screening (mean ABR: 1.9 [± 2.63]; median: 0.8; range: 0.0-7.2) than in the 4 subjects who had arthropathy at screening (0.5 [± 1.0]; median: 0; range: 0.0-2.0). None of the 4 subjects with arthropathy had non-joint BEs and none had spontaneous BEs. Similarly, the ABR of joint BEs was higher in the 7 subjects who did not have target joints at screening (mean ABR: 2.2 [± 2.71]; median: 2.0; range: 0.0-7.2) than in the 5 subjects who had target joints (mean ABR: 0.3 [± 0.7]; median: 0; range: 0.0-1.6). None of the 5 subjects with target joints at screening had non-joint BEs and none had spontaneous BEs. (see table below)

Table 11
Annualized Bleeding Rate in Subjects with 3+ Months of Prophylaxis Treatment by Bleeding Site, Cause and Baseline Characteristics (Study 251101: Full Analysis Set)

Age Group	Parameter	Category	Statistic	Site		Cause			All
				Joint[a]	Non-Joint[b]	Spontaneous	Injury	Unknown	
All Ages	Arthropathy at Screening	Yes	N	4	4	4	4	4	4
			Mean (Std)	0.5 (1.00)	0.0 (0.00)	0.0 (0.00)	0.5 (1.00)	0.0 (0.00)	0.5 (1.00)
			Median	0.0	0.0	0.0	0.0	0.0	0.0
			Q25 ; Q75	0.0 ; 1.0	0.0 ; 0.0	0.0 ; 0.0	0.0 ; 1.0	0.0 ; 0.0	0.0 ; 1.0
			Min ; Max	0.0 ; 2.0	0.0 ; 0.0	0.0 ; 0.0	0.0 ; 2.0	0.0 ; 0.0	0.0 ; 2.0
		No	N	19	19	19	19	19	19
			Mean (Std)	0.9 (1.90)	2.3 (2.49)	0.3 (0.72)	2.7 (3.06)	0.1 (0.48)	3.2 (3.26)
			Median	0.0	2.0	0.0	2.0	0.0	2.0
			Q25 ; Q75	0.0 ; 1.6	0.0 ; 3.6	0.0 ; 0.0	0.0 ; 4.2	0.0 ; 0.0	0.0 ; 5.4
			Min ; Max	0.0 ; 7.2	0.0 ; 8.2	0.0 ; 2.0	0.0 ; 10.8	0.0 ; 2.1	0.0 ; 10.8

Target Joints at Screening	Yes	N	5	5	5	5	5	5
		Mean (Std)	0.3 (0.70)	0.0 (0.00)	0.0 (0.00)	0.3 (0.70)	0.0 (0.00)	0.3 (0.70)
		Median	0.0	0.0	0.0	0.0	0.0	0.0
		Q25 ; Q75	0.0 ; 0.0	0.0 ; 0.0	0.0 ; 0.0	0.0 ; 0.0	0.0 ; 0.0	0.0 ; 0.0
		Min ; Max	0.0 ; 1.6	0.0 ; 0.0	0.0 ; 0.0	0.0 ; 1.6	0.0 ; 0.0	0.0 ; 1.6
	No	N	18	18	18	18	18	18
		Mean (Std)	1.0 (1.95)	2.4 (2.50)	0.3 (0.73)	2.9 (3.06)	0.1 (0.49)	3.4 (3.24)
		Median	0.0	2.0	0.0	2.0	0.0	2.0
		Q25 ; Q75	0.0 ; 1.8	0.0 ; 3.6	0.0 ; 0.0	0.0 ; 4.2	0.0 ; 0.0	0.0 ; 5.4
		Min ; Max	0.0 ; 7.2	0.0 ; 8.2	0.0 ; 2.0	0.0 ; 10.8	0.0 ; 2.1	0.0 ; 10.8
Baseline FIX at Screening	<1%	N	17	17	17	17	17	17
		Mean (Std)	0.6 (1.79)	2.2 (2.70)	0.2 (0.62)	2.6 (3.20)	0.0 (0.00)	2.8 (3.42)
		Median	0.0	1.9	0.0	1.9	0.0	1.9
		Q25 ; Q75	0.0 ; 0.0	0.0 ; 3.6	0.0 ; 0.0	0.0 ; 3.9	0.0 ; 0.0	0.0 ; 4.2
		Min ; Max	0.0 ; 7.2	0.0 ; 8.2	0.0 ; 2.0	0.0 ; 10.8	0.0 ; 0.0	0.0 ; 10.8
	1% - 2%	N	6	6	6	6	6	6
		Mean (Std)	1.4 (1.70)	1.0 (1.11)	0.3 (0.81)	1.7 (2.01)	0.3 (0.86)	2.4 (2.43)
		Median	1.0	1.0	0.0	1.0	0.0	2.0
		Q25 ; Q75	0.0 ; 2.0	0.0 ; 2.0	0.0 ; 0.0	0.0 ; 4.0	0.0 ; 0.0	0.0 ; 4.0
		Min ; Max	0.0 ; 4.2	0.0 ; 2.1	0.0 ; 2.0	0.0 ; 4.2	0.0 ; 2.1	0.0 ; 6.3

[a] Major joints: wrist, elbow, shoulder, hip, knee, ankle
[b] Soft tissue, muscle, body cavity, intracranial and other
[generated by 251101_csra_effic.sas]

Ancillary analyses

ABRs of joint and non-joint BEs, of spontaneous BEs and BEs caused by injury were also analyzed by baseline characteristics, such as arthropathy at screening, target joints at screening and baseline FIX at screening. Only subjects in the older age cohort had arthropathy (n=4) and/or target joints (n=5) at screening. None of the subjects <6 years of age had arthropathy or target joints at screening.

Of 23 subjects in the FAS, 17 had a baseline FIX activity level <1% at screening (9 subjects <6 years and 8 subjects 6 to <12 years of age) and 6 had a baseline FIX activity level 1-2% (2 subjects <6 years and 4 subjects 6 to <12 years of age).

In subjects with a baseline FIX activity level <1% (n=17), the mean ABR of joint BEs was 0.6 (± 1.79) (median: 0, range: 0.0-7.2) compared with a mean rate of 2.2 (± 2.70) (median: 1.9, range: 0.0-8.2) for non-joint BEs. In subjects with a baseline FIX activity level 1-2% (n=6), the mean ABR of joint BEs was 1.4 (± 1.70) (median: 1.0; range: 0.0-4.2) compared with a mean rate of 1.0 (± 1.11) (median: 1.0; range: 0.0-2.1) for non-joint BEs. Two subjects <6 years of age with a baseline FIX activity level 1-2% had no joint BEs.

Table 16: Summary of Efficacy for trial Summary of efficacy for trial 251101

A Phase 2/3, Prospective, Uncontrolled, Multicenter Study Evaluating Pharmacokinetics, Efficacy, Safety, and Immunogenicity in Previously Treated Paediatric Patients With Severe (FIX level <1%) or Moderately Severe (FIX level 1-2%) Haemophilia B

Study identifier	251101			
Design	This was a Phase 2/3, prospective, uncontrolled, multicenter study investigating the haemostatic efficacy, safety, immunogenicity and health related quality of life (HR QoL) of treatment with BAX326 over 6 months with twice weekly prophylactic infusions or at least 50 exposure days (EDs), whichever occurred last, in 23 paediatric subjects (PTPs). Before the start of the 6-month prophylactic treatment period, a PK evaluation was performed. There were 2 cohorts based on the age of the subjects: <6 years (n=11) and 6 to <12 years (n=12).			
	Duration of main phase:	6 months		
	Duration of Run-in phase:	not applicable		
	Duration of Extension phase:	not applicable		
Hypothesis	For all outcome measures descriptive statistics were to be presented by age stratum. Point estimates (mean or median) and 95% CIs were to be computed.			
Treatments groups	<6 years	BAX326 prophylactic treatment for 6 months or 50 EDs.; n=11		
	6-<12 years	BAX326 prophylactic treatment for 6 months or 50 EDs.; n=12		
Endpoints and definitions	Primary endpoint AEs	All possibly or probably related AEs, safety EP		
	Secondary endpoint ABR	Annualized bleeding rate		
	Secondary endpoint HEff	Overall haemostatic efficacy rating at resolution for BE		
	Secondary endpoint #Inf	Number of infusions per BE		
	Secondary endpoint Cons	Consumption IU/kg/year		

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	**Intent to treat**			
Descriptive statistics and estimate variability	**Treatment group**	**<6**	**6-<12**	**All**
	Number of subjects	11	12	23
	AEs	0	0	0
	ABR			
	mean	1.9	3.4	2.7
	median	2.0	1.8	2.0
	range	0.0; 5.4	0.0; 10.8	0.0; 10.8
	# of Bleeds	11	15	26
	HEff			
	excellent n(%)	9(81.8)	4(26.7)	13(50.0)
	good n(%)	2(18.2)	10(66.7)	12(46.2)

	fair n(%)		1(6.7)	1(3.8)
	# Inf			
	1	9	6	15
	2	1	7	8
	≥3	1	2	3
	Cons			
	mean	4,720.9	4,978.2	4.855.1
	median	4,722.2	4,532.6	4,910.5
	range	3,857 ; 5,602	3,822 ; 6,832	3,822-6,832

Study 251002

This was a phase 3, prospective, open-label, uncontrolled, multicenter study to evaluate the safety and efficacy of Rixubis in approximately 30 subjects with severe or moderately severe haemophilia B undergoing elective or emergency surgical, dental or other invasive procedures.

Methods

Study Participants

Inclusion and exclusion criteria were as in study 250901 with the following additional criteria:

Inclusion criteria:

- Subject is participating in either the BAX326 Pivotal Study (250901), the BAX326 Continuation Study (251001) or the BAX326 Paediatric Study (251101) requiring an emergency or elective major or minor surgical, dental, or other invasive procedure, and continues to meet eligibility criteria as outlined in the BAX326 pivotal, continuation, or pediatric study.
- For newly entering subjects, ie, subjects not participating in any other BAX326 clinical study, the following main inclusion criteria applied:
- Subject requires elective major surgery

Exclusion criteria:

- History of FIX inhibitors with a titer ⩾ 0.6 Bethesda Units (BU) (as determined by the Nijmegen modification of the Bethesda assay or the assay employed in the respective local laboratory) at any time prior to screening
- Detectable FIX inhibitor at screening, with a titer ⩾0.6 BU as determined by the Nijmegen modification of the Bethesda assay in the central laboratory
- Subject requires emergency surgery vi

Treatments

For major surgeries FIX levels should not fall below 80-100% of normal until wound healing is achieved. If the duration of the surgery is more than 4 hours, an additional bolus to increase the FIX level by 40%

may be administered after 4 hours. Depending on the subject's individual pharmacokinetics, FIX infusions needed to be repeated every 8 to 24 hours. Once adequate wound healing is achieved, FIX trough levels are to be maintained in a range of 30-60% of normal for 7 to 14 days, depending on the type and location of intervention. For orthopaedic surgery, trough levels of 20-40% for weeks 3-4 are still desirable. The World Federation of Haemophilia (WFH) recommends initial trough levels of 60-80% which should be maintained for at least 72 hours, and levels of 40-60% for days 4-6.

For minor surgeries FIX levels should not fall below 30-60% of normal for at least a day, depending on the nature and location of the intervention. Infusions are generally to be repeated every 24 hours, until healing is achieved.

The dosing guidance on how to maintain pre-infusion target plasma FIX levels and dosing frequency during the postoperative period is described below.

Dosing Guidance		
Type of Surgical Procedure	**FIX level required (%) (IU/dL)**	**Frequency of doses (hours) / Duration of therapy (days)**
Minor surgery including minor tooth extraction	30-60%	Every 24 hours, at least 1 day, until healing is achieved.
Major surgery	80-100% (pre- and postoperative)	Repeat infusion every 8-24 hours until adequate wound healing, then therapy for at least another 7 days to maintain a FIX activity of 30% to 60% (IU/dL)

Objectives

The primary objective was to evaluate the haemostatic efficacy and safety of BAX326 in the peri- and postoperative setting in subjects with severe (FIX level < 1%) or moderately severe (FIX level 1-2%) haemophilia B undergoing major or minor elective or emergency surgical, dental or other invasive procedures.

The secondary objectives were: To determine the actual intra- and postoperative blood loss at the end of surgery and until drain removal, if applicable, compared to the estimated volume of the expected average and maximum blood loss as predicted preoperatively by the operating surgeon; To determine the intra- and postoperative haemostatic efficacy at the end of the surgery, at the time of drain removal, if applicable, or at postoperative day 3 (approx. 72 h postoperatively) in case of major surgery and no drain employed, and at the time of discharge from the hospital on a scale of "excellent", "good", "fair" and "none"; To calculate the daily and total weight-adjusted dose of BAX326 per subject; To record the number of units and amount of blood product transfused; To record the development of inhibitory and total binding antibodies to FIX; To determine AEs related to BAX326; To determine the occurrence of thrombotic events.

Outcomes/endpoints

The intraoperative haemostatic efficacy was to be assessed by the operating surgeon according to the criteria shown below. The rating was to reflect the intraoperative blood loss as compared to the expected amount of blood loss estimated preoperatively for the type of procedure in a haemostatically normal individual.

Table 17: Intraoperative efficacy assessment

Intraoperative efficacy assessment	
Rating	**Criteria**

Intraoperative efficacy assessment	
Excellent	Intra-operative blood loss was less than or equal to that expected for the type of surgical procedure performed (≤100%)
Good	Intra-operative blood loss was up to 50% more than expected for the type of surgical procedure performed (101 -150%)
Fair	Intra-operative blood loss was more than 50% of that expected for the type of surgical procedure performed (>150%)
None	Uncontrolled haemorrhage that was the result of inadequate therapeutic response despite proper dosing, necessitating a change of FIX concentrate.

-

The postoperative haemostatic efficacy was also to be assessed by the operating surgeon, at the time of drain removal. The ratings were to reflect the volume in drain as compared to the volume estimated preoperatively for the type of procedure performed in a haemostatically normal individual. The rating criteria were as follows:

Table 18: Postoperative efficacy assessment at the time of the drain removal

Postoperative efficacy assessment at the time of the drain removal	
Rating	**Criteria**
Excellent	Volume in drain was less than or equal than that expected for the type of surgical procedure performed (≤100%)
Good	Volume in drain was up to 50% more than expected for the type of surgical procedure performed (101 -150%)
Fair	Volume in drain was more than 50% of that expected for the type of surgical procedure performed (>150%)
None	Uncontrolled bleeding that was the result of inadequate therapeutic response despite proper dosing, necessitating a change of FIX concentrate.

In the case of major surgery and where no drain was employed, the postoperative haemostatic efficacy was to be assessed by the operating surgeon on postoperative day 3 (approximately 72 h postoperatively).

Table 19: Postoperative efficacy assessment 72 hours postoperatively

Postoperative efficacy assessment 72 hours postoperatively	
Rating	**Criteria**
Excellent	Post-operative haemostasis achieved was as good or better than that expected for the type of surgical procedure performed

Postoperative efficacy assessment 72 hours postoperatively	
Good	Post-operative haemostasis achieved was as good as that expected for the type of surgical procedure performed
Fair	Post-operative haemostasis achieved was clearly less than optimal than that expected for the type of surgical procedure performed but was maintained without the need to change the FIX concentrate
None	Subject experienced uncontrolled bleeding that was the result of inadequate therapeutic response despite proper dosing, necessitating a change of FIX concentrate.

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On the day of discharge from hospital, the haemostatic efficacy was to be assessed by the investigator, i.e., haemophilia physician. The rating criteria were the same as for postoperative day 3 (see above).

Consumption of BAX326 during and after surgery

During the intraoperative period, the mean weight-adjusted dose in the PPAS was 188 IU/kg (range: 134-296 IU/kg) for major surgery (n=10), 200 IU/kg (range: 147-296 IU/kg) for orthopedic surgery (n=6) and 132 IU/kg (range: 55-203 IU/kg for minor surgery (n=3).

During the postoperative period, the mean weight-adjusted dose in the PPAS was 1,264 IU/kg (range: 415-2,965 IU/kg) for major surgery (n=10), 1,487 IU/kg (range: 829-2,965 IU/kg) for orthopedic surgery (n=6) and 291 IU/kg (range: 55-601 IU/kg) for minor surgery (n=3).

Blood Product use

Three subjects in the PPAS and 4 subjects in the FAS, who all underwent major (orthopedic surgery) received blood product transfusions, either in the form of packed red blood cells or fresh frozen plasma (FFP). The mean volume transfused was 558.7 mL in the PPAS (range: 520-600 mL). In the FAS, the mean volume transfused was 725.3 mL during the intraoperative period (range: 520-1225 mL) and 575 mL during the postoperative period.

Sample size

The sample size was not based on statistical considerations and was determined by the number of subjects participating in BAX326 Pivotal (250901), BAX326 Continuation (251001) and BAX326 Paediatric (251101) studies, who were undergoing major or minor elective or emergency surgical, dental or other invasive procedures. It was planned that approximately 30 elective or emergency surgical, dental or other invasive procedures would be performed in approximately 30 subjects. At least 10 of the procedures had to be major surgeries in 10 unique subjects. Additional subjects not participating in any of these studies could also be enrolled.

Randomisation

The study was not randomised.

Blinding (masking)

This was an open label study.

Statistical methods

The Full Analysis Set (FAS) comprised all subjects exposed to IP and who provide data suitable for the haemostatic efficacy analysis. The analysis in paediatric subjects < 12 years of age was to be performed separately, if applicable. The intra- and post-operative haemostatic efficacy assessment was to be summarized for the FAS by percentage of subjects in each efficacy categories ('excellent', 'good', 'fair' and 'none') at each time point. Actual intra- and postoperative blood loss, if applicable, and the difference from the expected average and maximum blood loss were to be summarized using descriptive statistics including median and range. The summary of average daily and total weight-adjusted dose of BAX326 per subject was to be provided using median and range. Units and amount (in mL) of blood product transfused was also to be presented descriptively.

A frequency count and the proportion of subjects with incidence of inhibitory and total binding antibodies to FIX were to be presented for the SAS. The occurrence of IP-related AEs and thrombotic events were to be evaluated descriptively.

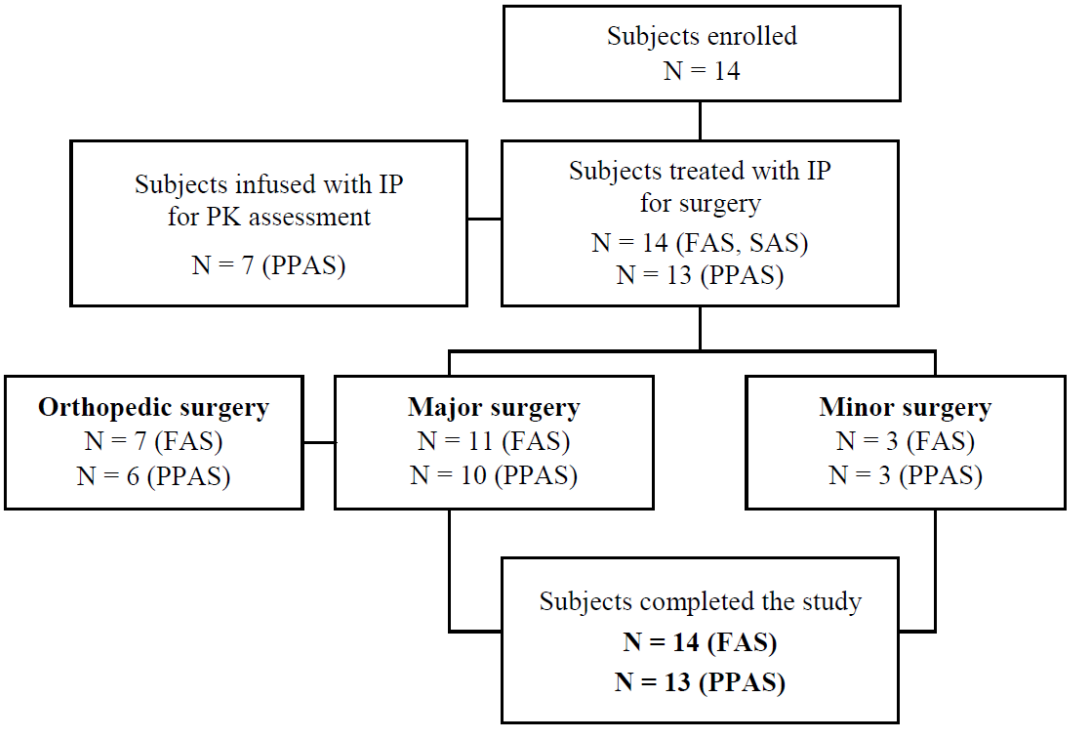
Results

Participant flow

Fourteen subjects have completed the protocol following treatment with BAX326 for surgery. All 14 subjects are included in the Full Analysis Set (FAS). Thirteen of 14 subjects had previously been treated in BAX326 Pivotal Study 250901.

Most protocol deviations were minor. Major deviations were reported for 7 subjects. The majority of these were of the category 'procedure not done' (eg, local lab result missing). The most serious deviation, which was a local breach of GCP and the protocol, was committed by an investigator who performed major surgery (total hip replacement) despite knowing that the FIX results from the local laboratory were unreliable and who based postoperative IP dosing and FIX monitoring on aPTT only. For this reason, the patient was not included in the PPAS.

Flow Chart for Study 251002



Recruitment

The 14 subjects included in the surgery study came from 5 study sites in 4 countries (Russia: 6 subjects from 2 sites, Bulgaria: 3 subjects, Poland: 3 subjects, Ukraine: 2 subjects). Thirteen of 14 subjects had previously been treated in BAX326 Pivotal Study 250901. Of those 13 subjects, 12 also received treatment in the BAX326 Continuation Study 251001.

Conduct of the study

The surgery study was initiated in December 2011 There have been 2 amendments to the study protocol, a global one mainly changing the design so that subjects taking part in the paediatric study (251101) could also be recruited into the surgery study and adding occurrence of thrombotic events as a safety endpoint and a local amendment specific to the UK.

Baseline data

The demographic and baseline characteristics are presented in Tables X and x.

Table 20: Demographic and Baseline Characteristics - Continuous Data (Study 251002)

Parameter	Statistic	Per-Protocol	Full	Safety
Age at Consent [years]	N	13	14	14
	Mean (Std)	39.4 (9.5)	39.0 (9.2)	39.0 (9.2)
	Median	38.0	38.0	38.0
	Q25 ; Q75	35.0 ; 42.0	34.0 ; 42.0	34.0 ; 42.0
	Min ; Max	19 ; 54	19 ; 54	19 ; 54
Weight [kg]	N	13	14	14
	Mean (Std)	74.6 (10.4)	73.8 (10.4)	73.8 (10.4)
	Median	75.0	75.0	75.0
	Q25 ; Q75	69.0 ; 80.0	64.1 ; 80.0	64.1 ; 80.0
	Min ; Max	56 ; 90	56 ; 90	56 ; 90
Height [cm]	N	13	14	14
	Mean (Std)	174.1 (3.4)	174.7 (4.0)	174.7 (4.0)
	Median	175.0	175.0	175.0
	Q25 ; Q75	172.0 ; 176.0	172.0 ; 176.0	172.0 ; 176.0
	Min ; Max	169 ; 180	169 ; 183	169 ; 183
Study Duration [days][a]	N	13	14	14
	Mean (Std)	44.5 (27.4)	42.5 (27.3)	42.5 (27.3)
	Median	40.0	39.0	39.0
	Q25 ; Q75	28.0 ; 58.0	17.0 ; 58.0	17.0 ; 58.0
	Min ; Max	4 ; 93	4 ; 93	4 ; 93

[a] Informed consent to study completion/termination

Eleven subjects had severe haemophilia B, with a FIX level <1%, and 3 subjects had moderately severe haemophilia B, with a FIX level 1-2%. FIX antigen levels were <1% in 6 (42.9%) subjects and ≥1% in 8 (57.1%) subjects, with levels ≥40% in 5 (35.7%) subjects. Gene mutations were diagnosed in 10 subjects; 7 had a missense, 2 a nonsense and 1 a frameshift mutation. All 14 subjects had arthropathy at screening (see below Table 21).

Table 21: Demographic and Baseline Characteristics - Categorical Data –Study 251002

Parameter	Category	Per-Protocol N = 13 n (%)	Full N = 14 n (%)	Safety N = 14 n (%)
Gender	Male	13 (100.0)	14 (100.0)	14 (100.0)
	Female	0 (0.0)	0 (0.0)	0 (0.0)
Age	≥16 yrs	13 (100.0)	14 (100.0)	14 (100.0)
Race	White	13 (100.0)	14 (100.0)	14 (100.0)
Ethnicity	Not Hispanic or Latino	5 (38.5)	5 (35.7)	5 (35.7)
	Not Reported	8 (61.5)	9 (64.3)	9 (64.3)
Gene Mutation	Missense	6 (46.2)	7 (50.0)	7 (50.0)
	Nonsense	2 (15.4)	2 (14.3)	2 (14.3)
	Frameshift	1 (7.7)	1 (7.1)	1 (7.1)
	No Mutation	1 (7.7)	1 (7.1)	1 (7.1)
	Not Reported	3 (23.1)	3 (21.4)	3 (21.4)
FIX Activity Level [%]	<1%	10 (76.9)	11 (78.6)	11 (78.6)
	1% - 2%	3 (23.1)	3 (21.4)	3 (21.4)
FIX Antigen Level [%]	<1%	6 (46.2)	6 (42.9)	6 (42.9)
	≥1%	7 (53.8)	8 (57.1)	8 (57.1)
	1% - 2%	2 (15.4)	2 (14.3)	2 (14.3)
	<2% - 5%	1 (7.7)	1 (7.1)	1 (7.1)
	≥40%	4 (30.8)	5 (35.7)	5 (35.7)
Arthropathy at Screening	Yes	13 (100.0)	14 (100.0)	14 (100.0)

Regarding the medical history classified by the system organ class (SOC), all 14 subjects had a history in the hematopoietic/lymphatic and musculoskeletal categories, due to the disease under investigation in this study (haemophilia B) and a diagnosis of haemophilic arthropathy. All 14 subjects also had a history of hepatitis A, B and/or C.

Numbers analysed

The Full Analysis Set (FAS) for the interim analysis comprises 14 subjects who underwent 14 surgeries of which 11 were major and 3 were minor surgeries. The Per Protocol Analysis Set (PPAS) comprises 13 surgeries of which 10 were major surgeries.

Outcomes and estimation

Of a total of 14 surgeries performed, 11 were major surgeries (of which 7 were orthopedic), and 3 were minor. Ten major surgeries (of which 6 were orthopedic) and 3 minor surgeries are included in the PPAS (n=13). Eleven major surgeries (of which 7 were orthopedic) and 3 minor surgeries are included in the FAS (n=14).

Intraoperative Blood Loss

In the PPAS (n=13), the mean intraoperative blood loss was 201.2 mL (range: 0-1100 mL). As expected, blood loss was higher in major (n=10) than in minor (n=3) surgeries (mean of 261.0 vs. 1.7 mL) and was highest in orthopedic (n=6) surgeries (420.0 mL, range: 20-1100). In terms of actual vs. predicted average/maximum intraoperative blood loss, actual blood loss during major surgery largely matched the predicted blood loss: 6/11 major surgeries matched the average predicted blood loss, 2/11 matched the maximum predicted blood loss, 2/11 were below the average predicted blood loss, and 1/11 (not included

in the PPAS due to major protocol deviation) was between the average predicted and maximum predicted blood loss. For all 3 minor surgeries, actual intraoperative blood loss was below the average predicted blood loss.

Postoperative Blood Loss

A total of 7 subjects, who all had major surgery (6 orthopedic, 1 non-orthopedic), had a drain placed. In the 6 subjects in the PPAS who had a drain placed, the mean postoperative blood loss was 703.5 mL (range: 11-1100 mL). Blood loss was slightly higher in orthopedic surgeries (n=5; mean: 842.0 mL, range: 500-1100 mL). In terms of actual vs. predicted average/maximum postoperative blood loss, actual blood loss was mostly higher than or equal to the maximum predicted blood loss: 4/7 major surgeries with drain placement were above the maximum predicted blood loss (including one surgery that is not part of the PPAS), 2/7 met the maximum predicted blood loss, and 1/7 was between the average predicted and maximum predicted blood loss. When analyzing the 4 major surgeries with an actual postoperative blood loss exceeding the maximum predicted blood loss, FIX levels on postoperative days 1-3 ranged between 34.3-40% for patient with a joint replacement and between 40-56% for a patient with a total hip replacement. The latter was excluded from the PPAS since the principal investigator (PI) dosed the patient according to aPTT values. In both patients, the drain was removed on postoperative day 3. For patient with a removal of residual nail from intramedullary nailing of left femur fracture, the postoperative actual blood loss exceeded the maximum predicted blood loss by 10 mL (810 vs 800 mL). The subject´s pre-infusion FIX levels were within the recommended range (84.5-91%). However, the initially predicted blood loss did not take into account the use of a tourniquet, and it was only decided intra-operatively to place a drain which was removed on postoperative day 1. The PI was then asked to retrospectively predict blood loss, taking into account the use of a tourniquet and the drain. Patient having a total knee replacement, had FIX levels ranging from 55.6-81.1%. The drain was removed on postoperative day 3; the postoperative actual blood loss exceeded the maximum predicted by 100 mL (1100 vs. 1000 mL).

Haemostatic Efficacy Assessment

All surgeries included in the intraoperative assessment (n=13 in the PPAS, n=14 in the FAS) had a rating of ‘excellent’. At drain removal, 50% of the ratings were ’excellent’and 50% were‘good’ (3/6 subjects in the PPAS and 3/7 subjects in the FAS had a rating of‘excellent’, and 3/6 subjects in the PPAS and 4/7 subjects in the FAS had a rating of ’good’). On postoperative day 3, all 6 surgeries in the PPAS/FAS where no drain was employed (including 4 major surgeries), had a rating of `excellent’. At discharge from hospital, most of the ratings were ‘excellent’ and the rest were ‘good’: 11/13 (84.6%) subjects in the PPAS and 11/14(78.6%) subjects in the FAS had a rating of ‘excellent’ and 2/13 subjects in the PPAS and 3/14 subjects in the FAS had a rating of ’good’.

Ancillary analyses

N/A

Summary of study 251002

The following table summarise the efficacy results from study 251002 supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 22: Summary of efficacy for trial 251002

BAX 326 (recombinant factor IX): A Phase 3, prospective, multicenter study evaluating efficacy and safety in previously treated patients with severe (FIX level < 1%) or moderately severe (FIX level 1-2%) haemophilia B undergoing surgical or other invasive procedures		
Study identifier	251002	
Design	This study is a Phase 3, prospective, open-label, uncontrolled, multicenter study to evaluate the safety and efficacy of BAX326 in approximately 30 subjects with severe or moderately severe haemophilia B undergoing elective or emergency surgical, dental or other invasive procedures.	
	Anticipated Duration of the study: Duration of treatment: Study Completion:	3 years Duration of participation per subject depended on the type of surgery and the intensity and duration of the haemostatic challenge, consistent with the study site's standards of care for surgical management of haemophilia B patients. study is ongoing
Hypothesis	The intra- (at the end of surgery) and postoperative (until drain removal, if applicable, or at postoperative day 3, i.e. approximately 72 hours, for major elective surgery where no drain is employed) haemostatic efficacy assessments and at the time of discharge from the hospital haemostatic efficacy assessment was to be summarized by percentage of subjects in each efficacy categories ("excellent, "good, "fair" and "none") at each time point. Actual intra- (at the end of surgery) and postoperative (until drain removal) blood loss , if applicable, and the differences from the expected average and maximum blood loss was to be summarized using descriptive statistics including median and range.	
Treatments groups	Major surgeries (all subjects >16 years)	N=11: 7 orthopedic, 2 abdominal,1 dental surgery and 1 excision of neurofibroma
	Minor surgeries (all subjects >16 years)	N=3: 2 dental surgeries and 1 intraarticular infusion
Endpoints and definitions	Primary endpoint IOPHEff	Intraoperative haemostatic efficacy assessment on a scale of "excellent", "good", "fair" and "none"
	Secondary endpoint IOPBL	Actual intraoperative blood loss compared to average and maximum blood loss predicted preoperatively by the operating surgeon
	Secondary endpoint POPHeff	Postoperative Haemostatic Efficacy Assessment at the time of discharge from the hospital on a scale of "excellent", "good", "fair" and "none"
	Secondary endpoint POPBL	Actual postoperative blood loss until drain removal, if applicable, compared to average and maximum blood loss predicted preoperatively by the operating surgeon
	Secondary endpoint BPU	Blood Product Use –volume transfused in ml
Results and Analysis		
Analysis description	**Primary Analysis**	
Analysis population and time point description	FAS (Full Analysis Set)	

Descriptive statistics and estimate variability

Treatment group	Major surgeries	Minor surgeries	All
Number of subjects	11	3	14
IOPHEff			
excellent n	11	3	14
IOPBL (ml)			
mean	309,0	1,7	236,8
median	100,0	2,0	35
Min; Max	10 ; 1100	0; 3	0; 1100

POPHEff

	Drain removal or postoperative day 3		
excellent n	7	2	9*
good n	4		4

***for one minor surgery NA**

POPBL(ml)

	N=7	NA	N=7
mean	784,4	NA	784,4
median	810,0	NA	810,0
Min; Max	11 ; 1270	NA	11 ; 1270

BPU (ml)

	Intra- N=4	Post-OP N=1	NA	Intra- N=4	Post-OP N=1
mean	725,3	575	NA	725,3	575
median	578	575	NA	578	575
Min; Max	520 ; 1225	575 ; 575	NA	520 ; 1225	575 ; 575

Analysis performed across trials (pooled analyses and meta-analysis)

A combined analysis of studies 250901 and 251101 was performed in order to increase precision of efficacy estimates, both for annualized bleeding rates and for haemostatic efficacy (response 'excellent/good' versus 'fair/none') in the treatment of bleeds. The Applicant has provided analyses using a negative binomial and a Poisson regression (annualized bleeding rates) and a logistic regression analysis considering the correlated data structure (haemostatic efficacy).

A pooled estimate (including a 95% CI) for the annualized bleeding rate of the prophylactic treatment arms of studies 250901 and 251101 (ignoring the on demand treatment arm from study 250901) was provided.

Table E135A
Analysis of ABR using Negative Binomial and Poisson Regression Model
(Study 250901 and 251101: Full Analysis Sets)

Model	Statistic	Prophylaxis 250901 12+ years N=56	Prophylaxis 251101 <12 years N=23	On-Demand 250901 12+ years N=14
Negative Binomial	ABR Estimate	4.24	2.60	33.80
	95% C.L.	3.11 ; 5.78	1.52 ; 4.46	19.76 ; 57.83
Poisson with Overdispersion	ABR Estimate	4.19	2.53	32.82
	95% C.L.	3.08 ; 5.70	1.35 ; 4.75	24.49 ; 43.97

Table E135B
Analysis of Hemostatic Efficacy Rate using Logistic Regression Model
(Study 250901 and 251101: Full Analysis Sets)

Statistic	Prophylaxis 250901 12+ years N=38	Prophylaxis 251101 <12 years N=17	On-Demand 250901 12+ years N=14	All N=69
Number of BEs included	114	26	134	274
P(Excellent or Good)	0.96	0.96	0.97	0.96
95% C.L.	0.91 ; 0.98	0.76 ; 0.99	0.88 ; 0.99	0.91 ; 0.98

Table E135C
Analysis of Hemostatic Efficacy Rate using Logistic Regression Model
(Study 251101: Full Analysis Set)

Statistic	Prophylaxis <6 years N=7	Prophylaxis 6 - <12 years N=10	Prophylaxis All Ages N=17
Number of BEs included	11	15	26
P(Excellent or Good)	NA[a]	NA[a]	0.96
95% C.L.	NA[a]	NA[a]	0.76 ; 0.99

The pooled estimates for the annualized bleeding rate of the prophylactic treatment arms of studies 250901 and 251101 are presented in the table below. The estimated annualized bleeding rates of the prophylactic treatment (95% CI) from negative binomial and poisson regression model are 3.77 (2.87; 4.95) and 3.72 (2.81; 4.91), respectively.

Table E135D
Analysis of ABR using Negative Binomial and Poisson Regression Model
(Study 250901 and 251101: Full Analysis Sets)

Model	Statistic	Prophylaxis N=79
Negative Binomial	ABR Estimate	3.77
	95% C.L.	2.87 ; 4.95
Poisson with Overdispersion	ABR Estimate	3.72
	95% C.L.	2.81 ; 4.91

Clinical studies in special populations
See paediatric study 251101

Supportive studies

N / A

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The efficacy of Rixubis in prophylaxis and control of bleeding in previously treated patients 12 years and older has been evaluated in the open-label, uncontrolled part of a combined phase 1/3 study 250901 in which a total of 73 male, previously treated patients (PTPs) between 12 and 59 years of age received Rixubis either for prophylaxis and/or for the treatment of bleeding episodes on an on-demand basis. All subjects had severe (factor IX level <1%) or moderately severe (factor IX level ≤2%) haemophilia B.

The safety and efficacy in the perioperative setting was evaluated in an ongoing phase 3 prospective, open-label, uncontrolled, multicenter study in male PTPs with severe and moderately severe haemophilia B using Rixubis (study 251002). The efficacy of Rixubis in prophylaxis and control of bleeding in PTPs below 12 years has been evaluated in a combined phase 2/3 study 251101. All subjects had severe (factor IX level <1%) or moderately severe (factor IX level ≤2%) haemophilia B.
The design of submitted clinical trials investigating efficacy (250901, 251101 and 251002) of BAX326 follows the requirements of the current guideline for recombinant FIX products (EMA/CHMP/BPWP/144552/2009).

The included patient population was multi-national and comprised previously treated children (0-12 years of age), adolescents and adults suffering from severe to moderately severe haemophilia B defined as FIX levels ≤2%. A total of 99 patients with haemophilia B had been exposed to BAX326 in the clinical development programme. Fifty-nine PTPs received Rixubis for prophylaxis. Fifty-six of these PTPs who received Rixubis for a minimum of 3 months (with at least 50EDs) were included in the efficacy evaluation for prophylaxis. An additional 14 PTPs received on demand treatment of bleeding episodes only until the last subject of the prophylactic cohort completed the study. Subjects in the on-demand cohort had to have at least 12 documented bleeding episodes requiring treatment within 12 months prior to enrolment.

The mean treatment duration in the on-demand cohort was 3.5±1.00 months (median 3.4, ranging from 1.2 to 5.1 months), the mean total annualised bleeding rate (ABR) was 33.9±17.37 with a median of 27.0, ranging from 12.9 to 73.1.

In the paediatric study, 23 male PTPs between 1.8 and 11.8 years (median age 7.10 years) with 11 patients < 6 years, received Rixubis for prophylaxis and control of bleeding episodes. All 23 subjects received prophylactic treatment with Rixubis for a minimum of 3 months (with at least 50EDs) and were included in the efficacy evaluation for prophylaxis.
In the on-going surgery study, 14 patients received preventive treatment during 14 surgeries (11 major, 3 minor).

The chosen endpoints are considered adequate for assessing efficacy of a new FIX product. Moreover, the endpoints are in accordance with the relevant guideline.

Treatment and dosage was in general in line with the "state of the art" recommendations as well as with the Core SmPC for FIX products. In trial 250901 (PTPs ≥12 years of age) the dose for prophylactic treatment was higher than recommended by the Guidelines for the Management of Haemophilia of the World Federation of Haemophilia or the Core SmPC for FIX products. However, the dose recommendations were comparable with those of another recombinant FIX product (BeneFix). Adults and adolescents could either receive a preventive dose regimen or an on-demand regimen in trial 250901. Children (0-12 years of age) could only receive preventive therapy.

Efficacy data and additional analyses

The annualized bleeding rate in the adult and adolescent population (≥12 years) was 4.26 in the prophylactic cohort. Appropriate statistical methodology has been applied to analyse results across the 2 trials and results were consistent and favourable for both efficacy endpoints. In the paediatric population the mean annualized bleeding rate for all 23 subjects with ≥3 months of prophylactic treatment was 2.7. Among adult and adolescent patients in the on-demand cohort the mean annualized bleeding rate was 33.87.

Most of the bleeding episodes were stopped with one or two infusions, 88.5% and 84.7% in the paediatric and adult/adolescent population, respectively. Furthermore, haemostatic efficacy of BAX326 was rated as excellent or good in 96.2 % of all bleeding events in the paediatric population and in 96% of all bleeding episodes in the adult/adolescent population. Generally, the consumption data observed across studies are regarded to be acceptable and reflect the current state of FIX-replacement-therapy.

The median ABR on prophylaxis with Rixubis for all bleeds was 2.0, for spontaneous bleeds 0.0, and for joint bleeds 0.0. 24 subjects (42.9%) experienced zero bleeds.

A total of 249 bleeding episodes were treated with Rixubis, of which 197 were joint bleeds and 52 non-joint bleeds (soft tissue, muscle, body cavity, intracranial and other). Of a total of 249 bleeding episodes, 163 were moderate, 71 were minor, and 15 were major. Treatment was individualised based on the severity, cause and site of bleed. Of the 249 bleeding episodes, the majority (211; 84.7%) were treated with 1-2 infusions. Haemostatic efficacy at resolution of bleed was rated excellent or good in 95.4% of all treated bleeding episodes.

In paediatric patients, the median ABR was 2.0, for spontaneous bleeds 0.0 and for joint bleeds 0.0. Nine subjects (39.1%) experienced zero bleeds. A total of 26 bleeding episodes were treated with Rixubis, of which 23 bleeds were due to injury, 2 spontaneous and 1 of unknown origin. 19 bleeds were non-joint (soft tissue, muscle, body cavity, intracranial and other) and 7 were joint bleeds of which 1 was a bleed into a target joint. Of the 26 bleeding episodes, 15 were minor, 9 moderate, and 2 major. Treatment was individualised based on the severity, cause and site of bleed. The majority (23; 88,5%) were treated

with 1-2 infusions. Haemostatic efficacy at resolution of a bleed was rated excellent or good in 96.2% of all treated bleeding episodes.

It is understandable that due to the small overall patient population available for investigation in haemophilia B each of the individual trials was of small sample size. As a consequence uncertainty on efficacy estimates necessarily remained large. In general, performing analyses across trials is a means to increase precision of estimates. This is though it is acknowledged, that each trial population has its own characteristics (e.g. age). The Applicant was asked to provide such analyses combining studies 250901 and 251101, both for annualized bleeding rates and for haemostatic efficacy (response 'excellent/good' versus 'fair/none') in the treatment of bleeds. In both cases, the requests regarding the additional statistical analyses on the individual studies were to be considered and point estimates as well as 95% confidence intervals were provided. Respective analyses have been performed and results showed favourable haemostatic efficacy as well as annualized bleeding rate. Submitted data are considered sufficient to demonstrate efficacy of BAX326 for prevention and treatment of bleeds in patients with Haemophilia B as well as efficacy during surgery. The guideline requirement to submit data of a minimum of 5 patients undergoing at least 10 surgical procedures (comprising major surgeries) is even exceeded with data of 14 patients undergoing surgical procedures of which 11 were major surgeries. Furthermore, the results are comparable with published data from other licensed FIX products (BeneFix, Haemonine).

The per-protocol efficacy analysis includes 13 surgeries performed in 13 patients
between 19 and 54 years of age undergoing major or minor surgical, dental or other surgical invasive procedures. Ten procedures were major including 6 orthopaedic and 1 dental surgery. Three procedures, including 2 dental extractions, were considered minor. Patients undergoing major surgeries had to perform a pharmacokinetic (PK) evaluation. All patients were dosed based on their most recent individual incremental recovery. The recommended initial loading dose of Rixubis was to ensure that during surgery, factor IX activity levels of 80-100% for major surgeries and 30-60% for minor surgeries were maintained. Rixubis was administered by bolus infusions.

Haemostasis was maintained throughout the study duration.
The rating scale for measuring intra-, postoperative blood loss and haemostatic efficacy was very strictly predefined and therefore the efficacy results can be considered highly accurate.

For 7 of 11 major surgeries, efficacy was rated as 'excellent', and for 4/11 major surgeries, as 'good`. For 7 major surgeries where drain was placed, postoperative haemostatic efficacy was rated at the time of drain removal as either 'excellent' or 'good'. All 4 major surgeries where no drain was placed had rating of 'excellent', and at the discharge from hospital, most of the ratings were 'excellent' and the rest were 'good'. In overall, the results of surgery study are satisfactory and comparable to published data for BeneFIX.

Subjects on prophylaxis reported statistically significant improvements between baseline and follow-up at approximately 6 months in the Physical Component Score ($p = 0.0189$) and the Bodily Pain ($p = 0.0146$) and Role Physical ($p = 0.0162$) domains of the SF-36 in addition to the EQ-5D VAS Score ($p = 0.005$). All other measures of HR QoL did not show significant changes over the course of the study in the subjects on prophylaxis. No significant differences in HR QoL were reported by the on-demand patients between baseline and follow-up.

The European Medicines Agency has waived the obligation to submit the results of studies with Rixubis in previously untreated patients in the treatment and prophylaxis of bleeding in haemophilia B (see section 4.2 of SmPC for information on paediatric use).
No Previously Untreated Patients (PUPs) were investigated through the clinical development plan. Despite the waiver granted by the PDCO for this population, collection of clinical data in PUP appears important and will be initiated via a registry (EUHASS, PedNet).Continuation study 251001 will further evaluate

haemostatic efficacy in 100 subjects who completed Study 250901 or Paediatric Study 251101 (see also RMP). Final report from the ongoing study 251002 is expected to be submitted by end 2014.

2.5.4. Conclusions on the clinical efficacy

Efficacy has been analysed for prophylaxis, on-demand treatment, treatment of breakthrough BE, and prophylaxis for surgical procedures. Study designs, selection and number of patients, assessment tools and results are in general adequate for supporting efficacy of the FIX product. Data have been reflected in the SmPC.

The available clinical data on nonacog gamma suggest that it is an efficacious new FIX product for the prevention and treatment of bleeds in previously treated patients suffering from Haemophilia B.

2.6. Clinical safety

In the clinical development programme, the safety of BAX326 is being assessed by monitoring the occurrence of AEs, by evaluating changes in clinical laboratory parameters and vital signs, and by assessing immunogenicity in terms of the development of FIX inhibitors, total binding FIX antibodies, and antibodies to CHO protein and rFurin.

Patient exposure
Data from 4 clinical trials contribute to the integrated analysis of safety:

- The pivotal study 250901 and the paediatric study 251101, both of which were completed..
- Study 251002, the surgery study, which is ongoing and of which an interim study report was provided.
- Study 251001, the continuation study, which is currently ongoing and where no separate report is available.

Overall, there are 99 unique subjects in the safety database: 73 subjects initially enrolled in Pivotal Study 250901, 23 subjects from Paediatric Study 251101 and 3 subjects enrolled directly (without prior enrolment in the Pivotal Study or Paediatric Study) in Surgery Study 251002.

Exposure to BAX326 FAS							
Parameter	**250901**			**251101**			**251002**
Number of patients	total	proph	OD	total	<6	6-<12	14[§] [3*; 25[+]]
	73	59	14	23	11	12	
Number of EDs/patient mean; median; range	46.9 (52) 5;83	56.3 (54) 50; 83	15.6 (14) 5;27	53.6 (53) 35;70	54 (53) 51;62	53.3 (51.5) 35;70	N/D
Total dose, IU	12,413,790	11,609,436	804,354	1,742,339	583,122	1,159,217	1,338,100
Total dose, IU/kg	177,980	166,208	11,772	70,772	34,202	36,570	17,895
Duration of study months median; range	7.69 3.2;12.3	7.85 6.9;12.3	4.80 3.2 6.7	7.52 4.3;10.6	7.75 7.2;8.9	7.36 4.3;10.6	39 days 4;93

§ Finished protocol until cut-off for interim CSR
*Directly enrolled into the surgery trial without previous participation in the pivotal or paediatric trial. [+] Total number of subjects treated in the surgery trial so far.

FAS Full Analysis Set
N/D No data available

Adverse events

Of the 337 AEs reported for subjects included in this integrated analysis, 327 (in 79 [79.8%] subjects) were non-serious.

Summary of the number of AEs by age group:

- <6 years - All (31) AEs were non-serious and not related: 22 of these AEs were rated as mild, 7 as moderate and 2 as severe.
- 6 to <12 years – 38 AEs in 10 subjects:
    - 4 not related SAEs in 3 subjects
    - 34 non-serious, not related AEs in 9 subjects
- 12 to <16 years – All (4) AEs were mild, non-serious and not related.
- ≥16 years- 264 AEs in 57 subjects:
    - 6 not related SAEs in 5 subjects
    - 258 non-serious in 57 subjects (252 AEs not related)

The majority of the non-serious AEs appear to have been related to mild infections or gastrointestinal disease, abnormal immunology tests (antibodies of indeterminate specificity in assays for FIX or rFurin) or arthralgia, a well-described complication of haemophilia, and not related to Rixubis.

Non-serious AEs using the Preferred Term which occurred in at least 4 subjects were evaluated for any pattern and compared with reference data from the general population not taking any medication, if available:

Anaemia: The Preferred Term included postoperative, haemorrhagic, hypochromic and normochromic anaemia. There were a total of 10 AEs of anaemia in 9/99 subjects recorded. The severity was moderate in 7 and mild in 3 cases. All AEs were considered not related by the investigator except one which is classified as not related by the sponsor. In one subject with a history of GI ulcer starting in 1999 two haemorrhagic anaemia occurred. Other cases of anaemia were the result of surgery or considered part of the underlying disease with repetitive bleeding episodes. A causal relationship to BAX326 can be ruled out.

Cough was reported in 6/99 subjects. In two subjects it occurred twice. The severity was mild except in one case and all AEs were considered as not related by the investigator. Cough started between 1 – 4 days following the last infusion of BAX326. In 5/8 AEs of cough there was a temporal association with infection/ infestation; in the other 3 cases, cough started between 1.9 and 3 days following the infusion. A causal relationship with BAX326 is unlikely.

Diarrhoea: This AE was reported 7 times in 4 subjects, in one subject occurring 4 times. They all were of mild severity and all were considered as not related by the investigator. In 3 subjects diarrhoea started 0 to 3 days after infusion of BAX326. The other subject experienced 4 AEs of diarrhoea in a 9 month period which occurred 0.4 to 1.1 days following infusion of BAX326. None of these AEs were connected to an infection/infestation and no overall pattern can be observed. The occurrence of diarrhoea in the study population with 4/99 is lower than that observed in the general population (5.5% mild, 0.8% moderate and 0.3% severe).

Headache: A total of 8 headaches were reported in 7/99 subjects of mild (5) or moderate (3) severity, all considered as not related by the investigator. The majority (5/7) started 2.3 to 5.8 days following the infusion of BAX326, in two cases it occurred between 0 to 0.4 days post-infusion. In 7 cases they were connected to an infection/infestation. The occurrence of headache in the study population is considerably lower than that reported for the general population which is 27.2% for mild severity, 9.5% for moderate and 1.3% for severe severity. The higher occurrence of headache in the general population and the variation in onset following the infusion of BAX326 precludes a relationship to BAX326.

Hypertension: There were 4 cases of hypertension reported in 4/99 subjects , all of mild severity and all considered a not related by the investigator. The start date varies from 0 – 2 days following the previous BAX326 infusion. Two of the subjects have obesity in their medical history as a potential risk factor. A relationship to BAX326 is unlikely given the low incidence and the different onset of time following the previous BAX326 infusion.

Pain in extremity: Four (4) subjects experienced pain in extremity in 5 cases consisting of “pain in hand” (2) in the same subject, “popliteal fossa pain” (1), “pain in the right forearm” (1) and “painful left foot” (1). The two instances of “pain in extremity” occurred in the same subject, apparently at the time of infusion. The severity and relationship of one AE was not provided by the investigator and thus conservatively classified as related by the sponsor. The other 3 cases occurred 1-3 days following the previous infusion of BAX326. Overall, a relationship to BAX326 can be ruled out given the different location and onset of the events. The two AEs of “pain in hand” are most likely due to injection of the product, but not BAX326 itself.

Pyrexia: There were a total of 10 AEs of pyrexia in 9/99 subjects, all of mild severity and all considered as not related by the investigator. The onset of the AEs varied between 0 – 4 days following the previous infusion of BAX326 and 2 AEs occurred at postoperative day 1 and 3 respectively. Three (3) AEs in 3 subjects were temporally related to infections/infestations.

Thrombocytosis: A total of 5 AEs of thrombocytosis were reported in 5/99 subjects. The severity was mild except in one subject and all thrombocytoses were considered as not related by the investigator to BAX326. Four (4) cases occurred following surgery at postoperative days 6 – 17. A reactive thrombocytosis is a common reaction following a surgical intervention and a relationship can be ruled out.

Adverse Events Considered Related to BAX326

Of the 337 AEs reported for the subjects included in this integrated analysis, the only AEs rated as related to BAX326 by the investigator or the sponsor were 6 non-serious AEs in 5 (5.1%) subjects. Two subjects were reported to have a positive (and transient) rFurin antibody test result (1:80), one subject experience 1 AE of haemorrhagic anaemia and 2 AEs of dysgeusia in 1 subject were also reported. Of these 6 AEs, 3 were rated as mild, 2 as moderate and 1 AE was of unknown severity and causality (pain in extremity). The latter was conservatively considered related.

Three of the AEs that were assessed by the investigator as related to BAX326 were reported in Pivotal Study 250901:

- Dysgeusia: 2 AEs in 1 subject, both considered mild
- Pain in extremity: 1 AE (at 1 day after BAX326 infusion, unknown severity and unknown relatedness and therefore conservatively rated in this analysis as related).

Two AEs considered by the investigator to be related to BAX326 were reported in Continuation Study 251001:

- Development of positive rFurin antibodies, titer 1:80: 2 AEs in 2 subjects, 1 considered of mild and 1 of moderate severity.

One AE of haemorrhagic anaemia was reported and assessed by the investigator as related to BAX326 in Surgery Study 251002. Baxter considers this AE to be unrelated to BAX326 for the following reasons: Anaemia is the result of surgery and postoperative hematoma and a common AE in the peri-operative setting. The intraoperative BL was lower than the predicted average BL and the postoperative BL matched the predicted average BL. No transfusions were required. The subject had adequate haemostatic coverage based on his FIX levels except on postoperative days 1 and 3.

No thrombotic events or severe allergic reactions were reported in any subject in the integrated analysis. In the paediatric study 251101, subject 130002 reported one adverse event of hypersensitivity, which was rated as mild and unlikely related and occurred 5.7 days after the last BAX326 infusion.

Serious adverse event / deaths / other significant events

Of the 337 AEs reported for subjects in this integrated analysis, 10 (in 8 [8.1%] unique subjects) were considered by the investigator to be serious. None of the SAEs were considered related to Rixubis. The SAEs, by preferred term were as follows:

☐ Duodenal ulcer haemorrhage: 1 SAE in 1 subject (severe) 251001

☐ Intestinal Obstruction: 1 SAE in 1 subject (severe) 250901

☐ Cervical vertebral fracture: 1 SAE in 1 subject (severe) 250901

☐ Traumatic hematoma: 1 SAE in 1 subject (severe) 250901

☐ Device Related Infection: 1 SAE in 1 subject (moderate) 251101

☐ Humerus Fracture: 1 SAE in 1 subject (moderate) 251101

☐ Convulsion: 1 SAE in 1 subject (moderate) 250901

☐ Hepatitis B core antibody positive: 1 SAE in 1 subject (mild) 250901

☐ Haemarthrosis: 1 SAE in 1 subject (mild) 251101

☐ Haemorrhage Subcutaneous: 1 SAE in 1 subject (mild) 251101

No death occurred in any of the studies.

Adverse Drug Reactions

The following adverse reactions have been identified during clinical development of Rixubis from 2 completed studies and 2 ongoing studies with 99 unique, male previously treated patients (PTPs) with haemophilia B receiving a total of 14,018 infusions.

Clinical Trials Adverse Reactions					
System Organ Class (SOC)	**Preferred MedDRA Term (Vers. 17.0)**	**Frequency per Patient[a] N=99**		**Frequency per Infusion[b] N=14,018**	
		Category	**Number of patients (Percentage)**	**Category**	**Number of occurrences (Percentage)**
NERVOUS SYSTEM DISORDERS	**Dysgeusia**	Common	1 (1.01%)	Rare	2 (0.014%)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	**Pain in extremity**	Common	1 (1.01%)	Very Rare	1 (0.007%)

Legend: Frequency is based upon the following scale: Very Common (≥1/10); Common (≥1/100–<1/10), Uncommon (≥1/1,000–<1/100), Rare (≥1/10,000–<1/1,000), Very Rare (<1/10,000)

Laboratory findings

Haematology and chemistry parameters were collected in the BAX326 clinical development program and assessed for clinical significance.

The haematology panel consists of complete blood count [haemoglobin, haematocrit, erythrocytes (i.e. red blood cell count), and leukocytes (i.e. white blood cell count)] with differential (i.e. basophils, eosinophils, lymphocytes, monocytes, neutrophils), mean corpuscular volume, mean corpuscular haemoglobin concentration, and platelet count.

The clinical chemistry panel consists of sodium, potassium, chloride, bicarbonate, total protein, albumin, ALT, AST, total bilirubin, alkaline phosphatase, blood urea nitrogen, creatinine, and glucose.

Two clinically significant haematology results were reported in 2 subjects 6-<12 years of age and 49 clinically significant results in 29 subjects ≥16 years of age. Two subjects had abnormal haematology values reported as mild unrelated AEs (low haemoglobin titer reported as mild anaemia and elevated platelet count reported as mild thrombocytosis).

Nine clinically significant clinical chemistry results were reported and 1 increased ALT value was reported as an AE in surgery 251102 study.

No viral laboratory changes were observed except 1 seroconversion to hepatitis B core antibody.

Furthermore, in trial 250901 thrombotic markers (prothrombin fragment 1.2, TAT complexes, D-dimer) were investigated. Thrombogenic marker levels were elevated in some subjects pre- and post-infusion of BAX326 as well as for BeneFIX. These abnormal results did not appear to correlate with infusion time or infused product.

No safety signals emerge from the laboratory findings.

Safety in special populations

Paediatric Use

The safety and efficacy of BAX326 was investigated in the Phase 2/3 Paediatric Study 251101. BAX326 was safe and well tolerated in 23 treated paediatric subjects (11 subjects <6 years and 12 subjects 6 to <12 years of age) during more than 3 months of prophylactic treatment (mean number of EDs to BAX326: 53.6 [± 6.11], median: 53.0, range: 35-70). No subjects developed inhibitors to FIX, and no subjects developed positive total binding antibodies to FIX, CHO proteins or to rFurin considered treatment related. There were no severe allergic reactions or thrombotic events. No significant treatment-related changes in laboratory values or vital signs were recorded.

A total of 48 AEs occurred in 17 (73.9%) subjects; 30 AEs occurred in 10 (83.3%) subjects in the 6-to-<12-year age cohort and 18 AEs occurred in 7 (63.6%) subjects in the <6-year age cohort. None of the AEs were considered treatment-related. Four unrelated SAEs (subcutaneous haemorrhage, humerus fracture, device-related infection, haemarthrosis) occurred in 3 subjects in the 6-to-<12-year age cohort; all 4 SAEs were resolved at the time of study completion.

Geriatric Use

Clinical studies of BAX326 do not include subjects aged 65 and over to determine whether they respond differently from younger subjects. As for all patients, dose selection for an elderly patient should be individualized. The use of BAX326 in patients ≥65 is expected to lead to a similar adverse event profile to that observed in the younger adults.

Clinical studies of BAX326 do not include subjects aged 65 or more, patients weighing more 120 kg or less than 35 kg, subjects with abnormal renal function, subjects with severe active or chronic liver disease and women. However, special populations have been investigated according to GL requirements.

Safety related to drug-drug interactions and other interactions

No interactions of BAX326 with other medicinal products are known.

Discontinuation due to adverse events

Two subjects were withdrawn from Pivotal Study 250901 due to unrelated SAEs requiring emergency treatment with another, non-IP FIX product rendering them ineligible for further participation in the pivotal study.

One subject who was screened into study 250901 performed a suicide attempt before receiving Rixubis and did not continue with the clinical trial.

All three discontinuations are not related to Rixubis.

One paediatric patient discontinued the 251101 trial because his guardian felt that nonacog gamma efficacy was insufficient.

Post marketing experience

N/A

2.6.1. Discussion on clinical safety

The evaluation of safety data in the pivotal, paediatric and surgery trial was done according to relevant guidelines. The size of the available safety database, 99 unique subjects of whom 23 are PTPs <12, exceeds guideline requirements.

A total of 337 AEs were reported in 80/99 (80.8%) patients treated with at least 1 infusion of Rixubis. The majority of AEs was non-serious (327/337) and considered unrelated to Rixubis (331/337). Overall, the majority of the non-serious AEs appear to have been related to mild infections or gastrointestinal diseases, abnormal immunology tests (indeterminate antibodies to FIX or rFurin) or arthralgia. Ten AEs in 8 (8.1%) subjects were considered serious, and all of these were classified as being unrelated to Rixubis. Six non-serious AEs, of dysgeusia (2), pain in extremity, development of positive rFurin antibodies (2) and haemorrhagic anaemia, reported in 5/99 patients were considered to be related to Rixubis.

No unexpected safety signals emerged throughout the clinical development programme. Rixubis appeared to be safe and well tolerated in paediatric and adult subjects during chronic administration of up to 83 exposure days as well as for the treatment of bleeding events and the peri- and postoperative use. No thromboembolic or severe allergic events occurred. No instances of inhibitor development were observed. Only 6 of 337 AEs were considered related to the administration of Rixubis in PTPs ≥12, while in PTPs <12 no related AEs were observed. In conclusion, the observed safety profile of Rixubis can be considered as benign.

Hypersensitivity or allergic reactions (which may include angioedema, burning and stinging at the infusion site, chills, flushing, generalised urticaria, headache, hives, hypotension, lethargy, nausea, restlessness, tachycardia, tightness of the chest, tingling, vomiting, wheezing) have been observed rarely and may in some cases progress to severe anaphylaxis (including shock). In some cases, these reactions have progressed to severe anaphylaxis, and they have occurred in close temporal association with development of factor IX inhibitors (see SmPC section 4.8). Rixubis is contra-indicated when Hypersensitivity to the active substance or to any of the excipients is present or known allergic reaction to hamster protein (See section 4.3 of the SmPC). Allergic-type hypersensitivity reactions have been reported with Rixubis. The product contains traces of hamster proteins. If symptoms of hypersensitivity occur, patients or their caregivers should be advised to discontinue use of the medicinal product immediately and contact their physician. Patients should be informed of the early signs of hypersensitivity reactions including hives, generalised urticaria, tightness of the chest, wheezing, hypotension, and anaphylaxis. The risk is highest during the early phases of initial exposure to factor IX concentrates in previously untreated patients (PUPs), in particular in patients with high-risk gene mutations. There have been reports in the literature showing an association between the occurrence of a factor IX inhibitor and allergic reactions, in particular in patients with a high-risk gene mutation. Therefore, patients experiencing allergic reactions should be evaluated for the presence of an inhibitor. In case of shock, standard medical treatment for shock should be implemented (see SmPC section 4.4).

The following general safety aspects associated with this class of products in the clinical setting of haemophilia are reflected in the SmPC sections 4.4 and 4.8:

Inhibitor development has not been observed with Rixubis as discussed above, however this is an important aspect of Factor IX treatment as patients with haemophilia B may develop neutralising antibodies (inhibitors) to factor IX. If such inhibitors occur, the condition will manifest itself as an insufficient clinical response. In such cases, it is recommended that a specialised haemophilia centre be contacted (see SmPC section 4.8). Precautions have been included in section 4.4 so that after repeated treatment with human coagulation factor IX (rDNA) products, patients should be monitored for the development of neutralising antibodies (inhibitors) that should be quantified in Bethesda Units (BU) using appropriate biological testing.

There have been reports in the literature showing a correlation between the occurrence of a factor IX inhibitor and allergic reactions. Therefore, patients experiencing allergic reactions should be evaluated for the presence of an inhibitor. It should be noted that patients with factor IX inhibitors may be at an increased risk of anaphylaxis with subsequent challenge with factor IX. Because of the risk of allergic reactions with factor IX concentrates, the initial administrations of factor IX should, according to the

treating physician's judgement, be performed under medical observation where proper medical care for allergic reactions could be provided.

Nephrotic syndrome has been reported following attempted immune tolerance induction in haemophilia B patients with factor IX inhibitors and a history of allergic reactions (see SmPC section 4.8).

Very rarely development of antibodies to hamster protein with related hypersensitivity reactions has been observed (see SmPC section 4.8).

There is a potential risk of thromboembolic episodes following the administration of factor IX products, with a higher risk for low purity preparations. The use of low purity factor IX products has been associated with instances of myocardial infarction, disseminated intravascular coagulation, venous thrombosis and pulmonary embolism. The use of high purity factor IX is rarely associated with such adverse reactions (see SmPC section 4.8). Because of the potential risk of thrombotic complications, clinical surveillance for early signs of thrombotic and consumptive coagulopathy should be initiated with appropriate biological testing when administering this product to patients with liver disease, to patients post-operatively, to new-born infants, or to patients at risk of thrombotic phenomena or DIC. In each of these situations, the benefit of treatment with Rixubis should be weighed against the risk of these complications (see SmPC section 4.4).

In patients with existing cardiovascular risk factors, substitution therapy with FIX may increase the cardiovascular risk(see SmPC section 4.4)..

After reconstitution this medicinal product contains 0.83 mmol (19 mg) sodium per vial. To be taken into consideration by patients on a controlled sodium diet (see SmPC section 4.4)..

Futhermore, if a central venous access device (CAVD) is required, risk of CVAD-related complications including local infections, bacteraemia and catheter site thrombosis should be considered(see SmPC section 4.4)..

Clinical studies of Rixubis did not include subjects aged 65 and over. It is not known whether they respond differently from younger subjects. As for all patients, dose selection for an elderly patient should be individualised(see SmPC section 4.4).

No interactions of human coagulation factor IX (rDNA) products with other medicinal products have been reported(see SmPC section 4.5).

As, animal reproduction studies have not been conducted with factor IX and based on the rare occurrence of haemophilia B in women, experience regarding the use of factor IX during pregnancy and breast-feeding is not available. Therefore, factor IX should be used during pregnancy and breast-feeding only if clearly indicated. There is no information on the effects of rFIX on fertility(see SmPC section 4.6).

Adverse drug reactions reported were dysgeusia (1.01%) and pain in extremity (1.01%).

The effects of higher than recommended doses of Rixubis have not been characterised (see SmPC section 4.9).

As discussed, frequency, type and severity of adverse reactions in children are expected to be the same as in adults. However, no data are available on previously untreated patients as only previously treated patients have been enrolled in the clinical studies; no immunogenicity investigation on inhibitor development was therefore made in this at risk population(see SmPC section 4.8).

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics section 4.8.

Further information also with regard to previously untreated patients and treatment in patients with

severe chronic hepatic disease will be collected through registries (e.g. the European Haemophilia Safety Surveillance EUHASS registry, PedNet registry) and will be submitted for review on an on-going basis.

2.6.2. Conclusions on the clinical safety

The extent and nature of the available safety data are considered adequate to support a marketing authorisation for Rixubis. Safety evaluation in general follows the currently valid Clinical Guideline. The presented results are considered to be acceptable.

Particular emphasis during the analysis and discussion of the safety data was given on immunogenicity and development of FIX antibodies. Further studies and registry data (see pharmacovigilance plan, RMP) will monitor safety issues of special interest.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

2.8. Risk Management Plan

The CHMP received the following PRAC Advice on the submitted Risk Management Plan:

PRAC Advice

Based on the PRAC review of the Risk Management Plan version 1, the PRAC considers by consensus that the risk management system for Nonacog gamma (Rixubis) for the treatment and prophylaxis of bleeding in patients with haemophilia B (congenital factor IX deficiency) is acceptable.

This advice is based on the following content of the Risk Management Plan:

- **Safety concerns**

Important Identified Risks	Hypersensitivity reactions (including reactions/antibodies to Chinese hamster ovary (CHO) protein)
Important Potential Risks	Inhibitor formation
	Lack of effect
	Thromboembolic events (e.g., DIC and fibrinolysis)
	Nephrotic syndrome following attempted ITI in haemophilia B patients with FIX inhibitors and a history of allergic reactions
Missing Information	No clinical data on use of RIXUBIS for ITI
	No clinical data on the use of RIXUBIS in geriatric patients
	No data on the use of RIXUBIS for continuous infusion
	The effects of RIXUBIS on male fertility have not been established in clinical trials
	No clinical data on the use of RIXUBIS in previously untreated patients (PUPs)
	No clinical data on the use of RIXUBIS in patients with severe chronic hepatic disease
	Insufficient data regarding the degree to which factor IX levels can be affected by the aPTT reagent in aPTT potency assay

- **Pharmacovigilance plans**

Study/Activity Type, title and category (1-3)	Objectives	Safety Concerns Addressed	Status (planned, started)	Date For Submission Of Interim Or Final Reports (planned or actual)
Study 251001 (Continuation study) (Category 3)	To further evaluate safety of BAX 326 in terms of IP-related AEs as well as clinically significant changes in routine laboratory parameters (hematology/ clinical chemistry) and vital signs To further evaluate the hemostatic efficacy of BAX326 in the prevention and routine prophylaxis of acute bleeding episodes using various prophylactic treatment regimens To further evaluate the hemostatic efficacy of BAX326 in the management of acute bleeding episodes To further evaluate immunogenicity for up to approximately 100 EDs to BAX326. To monitor incremental recovery (IR) of BAX326 over time To evaluate changes in health-related quality of life (HR QoL), Patient Activity Level and health resource use Exploratory: To correlate pre-infusion thrombin generation assay (TGA) parameters with preinfusion FIX levels and spontaneous breakthrough bleeds in a subset of subjects receiving twice weekly standard or modified	Inhibitor formation Hypersensitivity reactions (including reactions/antibodies to CHO protein) Thromboembolic events (e.g., DIC and fibrinolysis)	Ongoing	Final report: Approx. Q2 2016

	prophylactic treatment, including PK-tailored prophylaxis.			
Study 251002 (Surgery study) (Category 3)	To evaluate the hemostatic efficacy and safety of RIXUBIS in the peri- and postoperative setting in subjects with severe (FIX level < 1%) or moderately severe (FIX level ≤ 2%) hemophilia B undergoing major or minor elective or emergency surgical, dental or other invasive procedures	Not applicable	LSO 15 May 2014. iCSR available (dated 18 Oct 2012).	Final report: Approx October 2014
Participation in registries (e.g., European Haemophilia Safety Surveillance (EUHASS) registry PedNet registry) and review of the data provided by the registries to evaluate for a potential signal.	The EU HASS and PedNet registries serve to collect further safety information on previously untreated patients and patients with severe chronic hepatic disease	No clinical data on the use of RIXUBIS in previously untreated patients No clinical data on the use of RIXUBIS in patients with severe chronic hepatic disease	Planned- will start upon product launch in the EU.	Not applicable- data from registries will be monitored on an ongoing basis as part of Pharmacovigilance signal detection

- **Risk minimisation measures**

Safety Concern	Routine Risk Minimization Activities	Additional Risk Minimization Activities
Hypersensitivity reactions (including reactions/antibodies to CHO protein)	(Proposed) text in SmPC: Discussed in Section 4.3 of the EU SmPC, *Contraindications*. Discussed in Section 4.4 of the EU SmPC, *Special warnings and precautions for use* under the subsection *Hypersensitivity*. Discussed in Section 4.4 of the EU SmPC, *Special warnings and precautions for use* under the subsection *Inhibitors*. Discussed in Section 4.8 of the EU SmPC, *Undesirable effects*.	None
Inhibitor formation	(Proposed) text in SmPC: Discussed in Section 4.4 of the EU SmPC, *Special warnings and precautions for use* under the subsection *Inhibitors*. Discussed in Section 4.4 of the EU SmPC, *Special warnings and precautions for use* under the subsection *Nephrotic syndrome*. Discussed in Section 4.8 of the EU SmPC, *Undesirable effects*.	None
Lack of effect	(Proposed) text in SmPC: Discussed in Section 4.8 of the EU SmPC, *Undesirable effects*.	None
Thromboembolic events (e.g., DIC and fibrinolysis)	(Proposed) text in SmPC: Discussed in Section 4.4 of the EU SmPC, *Special warnings and precautions for use* under the subsections *Thromboembolism* and *Catheter-related complications*. Discussed in Section 4.8 of the EU SmPC, *Undesirable effects*.	None
Nephrotic syndrome following attempted immune tolerance induction (ITI) in	(Proposed) text in SmPC: Discussed in Section 4.4 of the EU SmPC, *Special warnings and precautions for use*	None

haemophilia B patients with FIX inhibitors and a history of allergic reactions	under the subsection *Nephrotic syndrome.* Discussed in Section 4.8 of the EU SmPC, *Undesirable effects.*	
No clinical data on use of RIXUBIS for ITI	(Proposed) text in SmPC: Discussed in Section 4.4 of the EU SmPC, *Special warnings and precautions for use* under the subsection *Nephrotic syndrome.* Discussed in Section 4.8 of the EU SmPC, *Undesirable effects.*	None
No clinical data on the use of RIXUBIS in geriatric patients	(Proposed) text in SmPC: Discussed in Section 4.4 of the EU SmPC, *Special warnings and precautions for use*: under the subsection *Elderly.*	None
No data on the use of RIXUBIS for continuous infusion	(Proposed) text in SmPC: Discussed in Section 4.2 of the EU SmPC, *Posology and method of administration* under the subsection *Continuous infusion.*	None
The effects of RIXUBIS on male fertility have not been established in clinical trials	(Proposed) text in SmPC: Discussed in Section 4.6 of the EU SmPC, *Fertility, pregnancy and lactation.*	None
No clinical data on the use of RIXUBIS in previously untreated patients (PUPs)	(Proposed) text in SmPC: Discussed in Section 4.2 of the EU SmPC, *Posology and method of administration.* Discussed in Section 4.8 of the EU SmPC, *Undesirable effects.*	None
No clinical data on the use of RIXUBIS in patients with severe chronic hepatic disease	(Proposed) text in SmPC: Discussed in Section 4.4 of the EU SmPC, *Special warnings and precautions for use.*	None
Insufficient data regarding the degree to which factor IX levels can be affected by the aPTT reagent in aPTT potency assay	(Proposed) text in SmPC: Discussed in Section 4.2 of the SmPC, *Posology and method of administration.*	None

The CHMP endorsed this advice without changes.

2.9. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the Guideline on the readability of the label and package leaflet of medicinal products for human use.

3. Benefit-Risk Balance

Benefits

Beneficial effects

In study 250901 the mean ABR in the prophylactic cohort (n=56) was 4.26 (median: 1.99; range: 0.0-23.4). Of a total of 249 BEs in the FAS, 115 BEs occurred during prophylactic treatment and 134 BEs occurred in the on-demand cohort (n=14). Haemostatic efficacy at resolution of bleed was rated 'excellent' in 41.0% and 'good' in 55.0% of all treated BEs (total of 96.0%). Only 2.0% of bleed treatments were rated as 'fair', and none had a rating of 'none'. For the remaining 2.0%, no efficacy ratings were provided. Of the 249 BEs, the majority (153; 61.4%) were treated with one infusion. Fifty (23.3%) BEs were treated with 2 infusions, and 38 (15.3%) BEs were treated with more than 3 infusions.

In the paediatric study 251101 the mean ABR for all 23 subjects was 2.7 (median: 2.0; range: 0.0-10.8). Fourteen subjects (7 subjects in each age cohort) had a total of 26 BEs which were treated. The haemostatic efficacy of BEs treated with Rixubis was mostly rated 'excellent' or 'good' (25 of 26 BEs; 96.2%), with only one rating of 'fair' and no ratings of 'none'. Fifteen of a total of 26 BEs were controlled with 1 infusion. A further 8 BEs were controlled with 2 infusions. Three BEs were treated with 3 or more infusions until resolution of bleed.

Efficacy of Rixubis in all surgeries included in the intraoperative assessment (n=14 in the FAS) had a rating of 'excellent'. On postoperative day 3, all 6 surgeries in the FAS where no drain was employed, had a rating of 'excellent" and at drain removal, and at discharge from hospital the ratings were either 'excellent' or 'good'. Four subjects in the FAS, who all underwent major (orthopaedic) surgery received blood product transfusions, either in the form of packed red blood cells (PRBC) or fresh frozen plasma (FFP). In the FAS, the mean volume transfused was 725.3 (334.8) mL during the intraoperative period (range: 520-1225 mL) and 575 mL during the postoperative period.

Submitted data are considered sufficient to demonstrate efficacy of BAX326 for prevention and treatment of bleeds in patients with Haemophilia B as well as efficacy during surgery. A pooled analysis of the two studies 250901 and 251101 for the annualized bleeding rate (for prophylactic treatment), resulting in an informative confidence interval, the estimated annualized bleeding rate lying within 2.8 and 5.0 bleeds per year.

Patients on prophylaxis reported improvements between baseline and follow-up at approximately 6 months in the HR-QoL measures such as physical Component Score and the Bodily Pain (and Role Physical domains of the SF-36 in addition to the EQ-5D VAS Score.

Uncertainty in the knowledge about the beneficial effects

There is no knowledge of the benefit of Rixubis in previously untreated patients, however this is reflected in the SmPC and it is anticipated that data will be collected on an on-going basis via registries (see discussion on clinical safety and RMP).

Risks

Unfavourable effects

The size of the safety database available at the moment exceeds guideline requirements, and the nature and frequency of the adverse events reported do not give rise to concern and do not reveal unexpected safety signals. Only a small proportion of observed AEs (6/337) were assessed as related to Rixubis by the

investigators: dysgeusia (2), pain in extremity (1), anti Furin antibodies (2), postoperative anaemia (1). No related SAEs occurred and importantly, no inhibitor development, thromboembolic event or severe allergic reactions were observed. However, 35 patients developed 43 instances of anti-RFIX and/or anti-rFurin antibodies throughout the clinical development program.

Uncertainty in the knowledge about the unfavourable effects

As for all diseases with a low incidence and prevalence, the size of the safety database pre-authorisation is quite small. However, the database will be expanded by data gathered in the ongoing extension study (251001) and surgery study (251002).

In view of the dependency of clotting assay results on the aPTT reagent used in the one-stage clotting assay (some reagents giving results 40% above the labelled potency), “underdosing of Rixubis” was discussed as a potential risk due to these highly variable results and to propose how to communicate this issue to the user, however it was considered that the variability of aPTT reagents in aPTT potency tests is not clearly understood and the root cause of the issue has not been elucidated. Therefore ‘Insufficient data regarding the degree to which factor IX levels can be affected by the aPTT reagent in aPTT potency assay’ has been included as Missing Information in the Risk Management Plan. Furthermore, a statement has been included in the SmPC (section 4.2) to make healthcare professionals aware that plasma factor IX activity results can be significantly affected by both the type of aPTT reagent and the reference standard used in the assay.

No data are available for previously untreated patients, and it is not known if the incidence of inhibitors or other adverse events will be comparable to PUPs treated with already licensed factor IX products. The PDCO has waived the need to conduct a PUP study in the approved PIP, because nonacog gamma is not considered a novel FIX product, however data from registries are expected to be collected post-authorisation (See Risk Management Plan).

Benefit-risk balance

Importance of favourable and unfavourable effects

Factor IX replacement with the aim of preventing and treating bleeding events is of paramount importance for patients with haemophilia B in order to enable work, school and social activities and to prevent long-term sequelae of repeat bleeds. Rixubis has been shown to be able to prevent and treat bleeding events as well as allow surgical intervention in paediatric and adult patients with haemophilia B. The observed unfavourable effects were generally benign and did not negatively impact the patients’ ability and willingness to continue treatment with Rixubis.

Benefit-risk balance

The beneficial effects of Rixubis clearly outweigh the unfavourable effects therefore the benefit-risk balance for Rixubis in the treatment and prophylaxis of bleeding in patients with haemophilia B (congenital factor IX deficiency) is positive.

Discussion on the benefit-risk balance

Haemophilia B is a sex-linked hereditary disorder of blood coagulation due to decreased levels of factor IX and results in profuse bleeding into joints, muscles or internal organs, either spontaneously or as a result of accidental or surgical trauma. By replacement therapy the plasma levels of factor IX is increased, thereby enabling a temporary correction of the factor deficiency and correction of the bleeding tendencies.

The submitted data are sufficient to provide relevant information on general safety and tolerability aspects and to demonstrate the efficacy of Rixubis in terms of its ability to raise factor IX levels and promote haemostasis and to stop as well as to prevent bleeding events as requested by the guideline. In patients on prophylaxis statistically significant improvements in health- related quality of life measures were also seen.

Post-marketing investigations from on-going trials and registries are expected to provide information on the treatment in PUPs and long-term safety including inhibitor development.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Rixubis in the treatment and prophylaxis of bleeding in patients with haemophilia B (congenital factor IX deficiency) is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription

Conditions and requirements of the Marketing Authorisation

- **Periodic Safety Update Reports**

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation. Subsequently, the marketing authorisation holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

If the dates for submission of a PSUR and the update of a RMP coincide, they can be submitted at the same time.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan PIP P/0159/2012 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.