# NON-INTERVENTIONAL (NI) STUDY REPORT

**PASS information**

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| **Title** | EUropean REgistry in Children below six years of age treated with BeneFIX - EUREKIX |
| **Version identifier of the final study report** | Final Version 3.0 |
| **Date of last version of the final study report** | 28 March 2018 |
| **EU PAS register number** | ENCEPP/SDPP/3788 |
| **Active substance** | Pharmacotherapeutic group: Antihaemorrhagic, blood coagulation factor IX; ATC code: B02BD09<br>Nonacog alfa |
| **Medicinal product** | rFIX (BeneFIX®) |
| **Product reference** | B1821046 |
| **Procedure number** | EMEA/H/C/000139 |
| **Marketing authorisation holder** | Pfizer Limited |
| **Joint PASS** | No |
| **Research question and objectives** | The objective of the study was to collect data in Europe regarding safety (primary endpoint) and efficacy (secondary endpoint) of treatment with rFIX (BeneFIX®) in children below 6 years of age treated in the routine clinical setting. |
| **Countries of study** | ITALY; SPAIN; SWEDEN; UNITED KINGDOM. |
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# 1 Abstract

**Title** EUropean REgistry in Children below six years of age treated with BeneFIX - *EUREKIX*

**Abstract date**: 29 January 2018

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**Keywords**

Paediatric, post-authorisation safety study (PASS), BeneFIX, Haemophilia B, registry

**Rationale and background**

Substitution of blood coagulation factor IX (FIX) is the treatment of choice for subjects with haemophilia B (congenital coagulation factor IX deficiency). BeneFIX is indicated for the treatment and prophylaxis of bleeding episodes in subjects with haemophilia B and surgical prophylaxis.

Whilst clinical trial data in adult subjects treated with BeneFIX is available for a relatively large number of subjects, data in paediatric subjects below 6 years of age is limited to date. A clinical study in 25 subjects below 6 years of age (Study 3030A1-301-WW) has demonstrated the safety and efficacy of BeneFIX in this age group. Due to small subject numbers in haemophilia B, registries are a valuable tool to assess safety and efficacy in routine clinical settings.

A European Registry on children below 6 years of age treated with BeneFIX would contribute valuable data related to the safety and efficacy of this type of treatment in children. This non-interventional study was designated as a voluntary PASS and was conducted by Pfizer.

**Research question and objectives**

The objective of the study is to collect data in EU regarding safety and efficacy of treatment with BeneFIX in children below 6 years of age treated in the routine clinical setting.

*Primary endpoint*

- Safety data related to treatment of haemophilia B with BeneFIX in children below 6 years of age treated in the routine clinical setting.

*Secondary endpoint*

- Efficacy data related to treatment of haemophilia B with BeneFIX in children below 6 years of age treated in the routine clinical setting.

**Study design**

This was a two-phase, non-interventional, multicentre registry study including retrospective (Phase I) and/or prospective (Phase II) data collection phases.

**Setting**

Subjects were eligible to take part in the retrospective data collection if they had been treated with BeneFIX for at least 12 months at an age below 6 years and were at time of consent not older than 8 years old. Subjects were eligible to participate in the prospective part of the study if they were able to accrue at least 12 months of data in the study before reaching the age of 6 years old. The maximum time of prospective follow-up was 24 months.

As a result of the data collection cut off scheduled for 31st July 2016, due to the completion of the retrospective phase and the completion of the prospective phase for the large majority of subjects included in the study, less than 12 months of prospective data was accrued for some subjects.

**Subjects and study size, including dropouts**

A statistical sample size calculation was not performed for this study since no statistical hypotheses were tested; thus, there was no need to determine statistical power. Approximately 50 subjects were planned for inclusion in this study.

**Variables and data sources**

- Subject baseline demographic data and disease/treatment history were collected. In addition to this, determinants for safety (i.e. adverse drug reactions [ADRs] and serious adverse drug reactions [SADRs] in the retrospective phase; treatment-emergent adverse events [TEAEs] and treatment-emergent serious adverse events [TESAEs] in the prospective phase; and events of special interest [ESIs] in both the retrospective and prospective phases) as well as for efficacy (i.e. annualized bleeding rates, responses to on-demand treatment with BeneFIX for bleeds of subjects receiving on-demand and prophylactic regimens, incidence of less-than expected therapeutic effect [LETE] and lack of effect)
- Data were collected from the subject's treatment records and from their treatment diaries. All data collected had been assessed in routine clinical practice. Due to the non-interventional nature of this study, no additional visits or procedures were requested for the study.

**Results**

This is a two-phase, non-interventional and multicentre registry study including retrospective and prospective data collection phases.

Retrospective Phase

From the 38 paediatric subjects who were eligible for inclusion in the retrospective phase 37 subjects were included. One subject was classified as a screening failure due to being nine years old at the time of enrolment. All of the subjects that entered the study were male, 34 (91.9%) were Caucasian, and the mean (SD) age of subjects was 5.0 (2.1) years old. Severe haemophilia B was reported for 25 (67.6%) subjects and BeneFIX was used as the first treatment for 32 (86.5%) subjects. The mean (SD) age at which the subject

experienced their first bleed was 0.8 (0.8) years old, and the mean (SD) age for the first use of BeneFIX was 1.1 (0.8) years old.

Two subjects receiving an ITI regimen were excluded from all efficacy analysis. The remaining 35 subjects were categorised as having a "prophylaxis" (24 [64.9%]) or "on-demand" (11 [29.7%]) regimen if they were receiving the regimen for more than 50% of the study period. The mean (95% confidence interval [CI]) annualised bleeding rate (ABR) was 3.4 (2.5, 4.4). The mean (95% CI) ABR for the 24 (64.9%) subjects mainly receiving a prophylaxis regimen was 3.7 (2.7, 4.7). The mean (95% CI) ABR for the 11 (29.7%) subjects receiving an on-demand regimen was 3.0 (0.8, 5.1). In all 35 subjects, the mean (95% CI) ABR related to traumatic bleeds was 2.2 (1.5, 2.9); the mean (95% CI) ABR related to spontaneous bleeds was 0.5 (0.3, 0.7). For soft tissue/muscle bleeds and joint bleeds the mean (95% CI) ABR were 1.1 (0.6, 1.6) and 0.6 (0.4, 0.8), respectively. The mean (95% CI) ABR for central nervous system (CNS) bleeds was 0.1 (0.0, 0.1).

The response to on-demand treatment with BeneFIX reported by subjects receiving on-demand or prophylactic treatment regimens (n=35) was mostly rated as *Excellent* by the investigator (n=33; 94.2%). For treatment of bleeds reported by the 24 subjects categorised as having a prophylaxis regimen, 23 (95.8%) subjects were rated to have an excellent response. Likewise, the majority (n=10, 90.9%) of subjects categorised as having on-demand regimen were reported to have excellent responses.

One less-than-expected therapeutic effect (LETE) occurred in the retrospective phase (a spontaneous bleed related to prophylaxis treatment). There were three cases of a lack of effect documented in two (5.4%) subjects, all mild in severity. Two events were considered to be related to BeneFIX and in all three cases, the dose of BeneFIX was increased and no further action with the treatment was required. Lastly, mean (95% CI) FIX activity (n=9) was 26.0 (5.2, 46.7) IU/dL and the mean (95% CI) FIX activity recovery (n=15) was 1.4 (0.0, 3.0) IU/dL of FIX:C increase per IU/kg of BeneFIX.

All 37 subjects were included in the safety population. In this retrospective phase, only those adverse events related to BeneFIX (ADRs and ESIs) were recorded. There were two ADRs reported (ecchymosis and skin haemorrhage), both experienced in one (2.7%) subject. Both ADRs were of mild severity and resolved by the end of this study phase. None of them was considered as serious. Four ESIs were experienced by one (2.7%) subject – all were considered to be serious (SESIs). The subject experienced hypersensitivity of mild severity on four occasions; no action with the treatment was taken and all ESIs were resolved by the end of the study phase. No deaths were reported. No subject experienced an ADR or ESI that led to permanent treatment discontinuation.

Prospective Phase

From the 27 subjects who were eligible for inclusion in the prospective phase, 26 subjects were included with 17 (65.4%) subjects continuing from the retrospective phase of the study. One subject was classified as a screening failure due to being older than five years old at the time of inclusion. All subjects were male, predominantly Caucasian (n=23; [88.5%]), with a mean (SD) age of 2.5 (1.1) years old. Twenty (76.9%) subjects suffered from severe haemophilia B and BeneFIX was used as the first treatment for 23 (88.5%) of subjects. The mean (SD) age of first bleed was 0.6 (0.4) years old, and the mean (SD) age at which the subject was first exposed to BeneFIX was 0.9 (0.7) years old.

Two subjects receiving an ITI regimen were excluded from all efficacy analysis. The mean (95% CI) ABR for the 24 subjects included in the efficacy analysis was 2.9 (0.9, 5.0) times per year. The mean (95% CI) ABR for the 19 subjects mainly receiving a prophylaxis

regimen was 2.9 (0.3, 5.4). The mean (95% CI) ABR for the five subjects receiving an on-demand regimen was 3.3 (0.0, 6.8). In all 24 subjects, the mean (95% CI) ABR related to traumatic bleeds was 2.2 (0.3, 4.2). The mean (95% CI) ABR related to spontaneous bleeds was 0.5 (0.1, 1.0). For soft tissue/muscle bleeds and joint bleeds the mean (95% CI) ABR were 0.9 (0.2, 1.7) and 0.4 (0.1, 0.8), respectively. The mean (95% CI) ABR for CNS bleeds was 0.1 (0.0, 0.1).

The response to on-demand treatment with BeneFIX was mostly rated as *Excellent* by the investigator (n=16; 66.7%); treatment of bleeds for subjects prescribed prophylaxis regimens, as well as bleeds treated for subjects prescribed on-demand regimens had the majority of collected responses rated as *Excellent*, 63.2% (n=12) and 80.0% (n=4).

No LETEs or lack of effect cases were reported for subjects in the prospective phase. Mean FIX activity was 2.9 (1.2, 4.6) IU/dL, and mean FIX activity recovery was 0.5 (0.2, 0.8) IU/dL of FIX:C increase per IU/kg of BeneFIX. With regard to the daily living impact, the median (min - max) total number of days missed from work by parents or caregivers (n=10) was 0 (0 – 5) days. The median (min - max) total number of days when the subject was affected in daily activities by the disease (n=9) was 0 (0 - 5) days.

In the safety analysis, 52 TEAEs were recorded for 19 (73.1%) subjects. The most common TEAEs by SOC were: Injury, oisoning and procedural complications (n=9 [34.6%]); Infection and infestations (n=6 [23.1%]); General disorders and administration site conditions (n=5 [19.2%]); Gastrointestinal disorders (n=3 [11.5%],) and Blood and lymphatic system disorders (n=3 [11.5%]. The most common (> 10%) TEAEs by PT were Head injury (n=9 [34.6%]) and Pyrexia (n=4 [15.4%]). Three (11.5%) subjects had at least one TEAE related to BeneFIX, (all n=1 [3.9%]): Cyanosis, Fatigue, Nephrotic syndrome, Cough, Blister, Erythema and Flushing.

Twenty-five TESAEs were reported by 14 (53.8%) subjects. The most common TESAE by PT was Head injury (n=6; 23.1%). The incidence by PT of all the other TESAE reported (Anaemia, Neutrophilia, Cyanosis, Mouth haemorrhage, Tongue haemorrhage, Fatigue, Oedema peripheral, Pyrexia, Lower respiratory tract infection, Contusion, Excoriation, Seizure, Device issue, Nephrotic syndrome, Cough, Blister, Erythema, Flushing and Hematoma) was of 3.9% (n=1). Three (11.5%) subjects reported seven treatment-related TESAEs during the prospective phase of the study, all n=1 (3.9%): Cyanosis, Fatigue, Nephrotic syndrome, Cough, Blister, Erythema and Flushing.

The dose was increased in seven (26.9%) subjects due to a TESAE. Additionally, three (11.5%) subjects permanently discontinued treatment due to a TESAE; one (3.8%) subject due to Cough, Flushing and Fatigue of mild severity and Factor IX inhibition of severe severity; one (3.8%) due to a Nephrotic syndrome of moderate severity; and one (3.8%) due to Hypersensitivity (serious ESI described below), Erythema and Blisters of unknown severity.

A total of four ESIs were experienced by three (11.5%) subjects, all of which were considered to be serious (SESIs). One (3.6%) subject experienced Factor IX inhibition of severe severity; one subject experienced severe Hypersensitivity, one subject experienced mild Hypersensitivity; and one subject experienced Hypersensitivity of unknown severity. All ESIs were considered treatment related. Two (7.7%) subjects permanently discontinued treatment due to an ESI (Factor IX inhibition and Hypersensitivity). No deaths were reported.

**Discussion**

The primary endpoint of this two phase non-interventional, multicentre registry study which included a retrospective and prospective phase was to assess the safety of BeneFIX in subjects below six years of age.

In the retrospective phase, ADRs and ESIs related to BeneFIX were collected. Two ADRs and four SESIs, all of mild severity, were recorded. By contrast, in the prospective phase, all TEAEs and ESIs were recorded, regardless of relation to BeneFIX. A total of 52 TEAEs, 25 TESAEs and 4 ESIs were recorded in this phase. Differences in data collection methodologies and time of follow-up make direct comparisons between phases challenging. Overall, the adverse events assessed as related to treatment with BeneFIX and reported in each of the phases in this study were consistent with the profile observed in children and adults exposed to the product in other studies conducted by the marketing authorisation holder in addition to the information accrued with BeneFIX through market exposure. No new safety concerns were detected in this study with a safety profile commensurate with that communicated through the product EU SmPC.[1]

The efficacy secondary objective of this study was achieved by assessing ABRs, responses to treatment regimens, the incidence of LETEs and the lack of treatment effect. Overall ABR rates in both phases of this study were similar to previous studies. Unlike previous BeneFIX studies, differences in the on-demand ABR between prophylaxis vs. on-demand regimens were observed to be substantially smaller; most likely attributable to subjects being able to transition between regimens and thus obscuring underlying differences. Responses to treatment regimens, LETE incidence, and the lack of treatment effect were in alignment with prior BeneFIX studies. In agreement with previous BeneFIX studies in adults and children, on-demand infusion responses in subjects receiving either a prophylaxis or on-demand treatment regimen were considered to be Excellent or Good in both phases of this study. The paucity of prior data for LETEs or lack of effect endpoints prevents any direct comparison or any substantive conclusions.

As for any study, there are limitations inherent to the study design. In the retrospective phase of the study, safety and efficacy were measured based on medical history, thus giving limited control over the data collection as it was gathered retrospectively. Furthermore, the voluntary participation of investigators constituted a selection bias observed for this type of study. Another potential selection bias classically associated with non-interventional studies was subject selection. Voluntary or involuntary selection of subjects in a study by investigators is inevitable, but this bias was limited by systematic attempts of the investigators to enrol subjects in the study. The pragmatic nature of this study, which involved routine clinical care management practices, complicated the collection of follow-up data and may have increased the number of subjects lost to follow-up. Differential losses to follow up can also lead to bias in retrospective cohort studies especially as the retrospective to prospective comparison were not performed in the same subjects throughout the study, rather both groups were analysed separately.

The results from this two-phase study included a paediatric cohort of subjects of varying haemophilia B severity from four EU countries. The wide eligibility criteria, with only those receiving treatment other than BeneFIX being excluded from participation, affords a high degree of external validity in predominantly Caucasian nations with equivalent standards of life. Nevertheless, the absence of ethnic diversity and healthcare system models included in this study are important limitations for the generalisability of these results to the global paediatric population of haemophilia B sufferers.

# 2 List of abbreviations

| Abbreviation | Definition |
|---|---|
| ABR | Annualized Bleeding Rate |
| ADR | Adverse Drug Reaction |
| AEs | Adverse Events |
| CHO | Chinese Hamster Ovary |
| CI | Confidence Interval |
| CNS | Central Nervous System |
| eCRF | Electronic Case Report Form |
| EMA | European Medicines Agency |
| ESI | Event of Special Interest |
| EU | European Union |
| FASP | Full Analysis Set for Prospective data |
| FASR | Full Analysis Set for Retrospective data |
| FIX | Factor IX |
| rFIX | Recombinant factor IX |
| IDR | Incidence Density Rate |
| IEC | Independent Ethics Committee |
| INN | International Non-proprietary Names |
| IRB | Institutional Review Board |
| ITI | Immune Tolerance Induction |
| IU | International Unit |
| LETE | Less Than Expected Therapeutic Effect |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MTP | Minimally Treated Subjects |
| PASS | Post-Authorisation Safety Study |
| PPP | Per Protocol analysis set for Prospective data |
| PPR | Per Protocol analysis set for Retrospective data |
| PT | Preferred term |
| PTP | Previously Treated Patient |

| | |
|---|---|
| PUP | Previously Untreated Patient |
| RBC | Red Blood Cells |
| SADR | Serious Adverse Drug Reaction |
| SAE | Serious Adverse Event |
| SASP | Safety Analysis Set for Prospective data |
| SASR | Safety Analysis Set for Retrospective data |
| SESI | Serious Event of Special Interest |
| SOC | System Organ Class |
| SmPC | Summary of Product Characteristics |
| TEAE | Treatment-Emerging Adverse Event |
| TESAE | Treatment-Emergent Serious Adverse Event |
| TEHE | Treatment-Emerging Haemophilia Event |
| UAT | User Acceptance Testing |

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# 5 Milestones

**Table 1 Study Milestones**

| Milestone | Planned date | Actual date | Comments |
|---|---|---|---|
| Start of data collection | 12 JUL 2013 | 12 JUL 2013 | Not applicable |
| End of data collection | 31 JUL 2016 | 31 DEC 2016 | Delayed data entry postponed the end of data collection |
| First Ethic Committee Approval | DEC 2012 | 19 DEC 2012 | Not applicable |
| Registration in the EU PAS register | 09 APR 2013 | 09 APR 2013 | Not applicable |
| Final report of study results | MARCH 2017 | 28 MAR 2018 | Delayed due to discussions related to changes in the planned analysis results |

# 6 Rationale and background

## 6.1 Background

Substitution of blood coagulation factor IX (FIX) is the treatment of choice for subjects with haemophilia B (congenital FIX deficiency).

BeneFIX (Nonacog alfa) is indicated for treatment and prophylaxis of bleeding in subjects with haemophilia B.

BeneFIX contains recombinant coagulation FIX (INN = nonacog alfa). Nonacog alfa is a purified protein that has 415 amino acids in a single chain. It has a primary amino acid sequence that is comparable to the Ala148 allelic form of plasma-derived FIX, and some post-translational modifications of the recombinant molecule are different from those of the plasma-derived molecule. Recombinant coagulation FIX is a glycoprotein that is secreted by genetically engineered mammalian cells derived from a Chinese hamster ovary (CHO) cell line.

The dosage and duration of the substitution therapy depends on the severity of the FIX deficiency, the location and extent of bleeding, and the subject's clinical condition. The amount to be administered and the frequency of administration should always be oriented to the clinical effectiveness in the individual case. Factor IX products rarely require to be administered more than once daily.

One International Unit (IU) of FIX activity is equivalent to that quantity of FIX in one ml of normal human plasma. Estimation of the required dose of BeneFIX can be based on the finding that one unit of FIX activity per kg body weight is expected to increase the circulating level of FIX, an average of 0.8 IU/dL (range from 0.4 to 1.4 IU/dL) in adolescents and adults. Pharmacokinetics must be assessed regularly in each subject and posology adjusted accordingly.

| | | | | | | |
|---|---|---|---|---|---|---|
| Number of FIX IU required | = | body weight (in kg) | · | desired FIX increase (%) or (IU/dL) | · | reciprocal of observed recovery |

For a recovery 0.8 IU/dL (average increase of FIX in adolescents and adults), the formula reads:

| | | | | | | |
|---|---|---|---|---|---|---|
| Number of FIX IU required | = | body weight (in kg) | · | desired FIX increase (%) or (IU/dL) | · | 1.3 IU/kg |

During the course of treatment, appropriate determination of FIX levels is advised to guide the dose to be administered and the frequency of repeated infusions. In the case of major surgical interventions in particular, precise monitoring of the substitution therapy by means of coagulation analysis (plasma FIX activity) is indispensable. Individual subjects may vary in their response to FIX, achieving different levels of in vivo recovery and demonstrating different half-lives.

Subjects should be monitored for the development of FIX inhibitors. If the expected FIX activity plasma levels are not attained, or if bleeding is not controlled with an appropriate dose, biological testing should be performed to determine if factor IX inhibition is present.

For long term prophylaxis against bleeding in subjects with severe haemophilia B, BeneFIX may be administered. In a clinical study for routine secondary prophylaxis the average dose for previously treated subjects (PTP) was 40 IU/kg (range 13 to 78 IU/kg) at intervals of 3 to 4 days. In younger subjects, shorter dosage intervals or higher doses may be necessary.

For further information on BeneFIX please refer to the current version of the Summary of Product Characteristics (SmPC).

Randomized controlled trials have demonstrated the efficacy and safety of BeneFIX for prophylactic treatment, on-demand treatment and surgery.[2–4]

Study 3030A1-301-WW (hereinafter referenced as study 301-WW) was an open-label, single-arm, safety and efficacy study of recombinant FIX (rFIX, BeneFIX) in children less than 6 years of age with severe haemophilia B.[5] This study provides safety, efficacy, and pharmacokinetic data in paediatric subjects. A total of 25 subjects at 19 treatment centres participated in this study. Subjects had to be less than 5 years of age (to complete the study before attainment of age 6) with severe haemophilia B (FIX: C≤1%), and with no detectable FIX inhibitor (defined as ≥0.6 Bethesda Units) or history of inhibitor. At least 6 subjects were to have had minimal (≤20 exposure days) or no prior exposure to BeneFIX.

BeneFIX was efficacious in the treatment of children less than 6 years of age with severe haemophilia B who were previously untreated subjects (PUPs), minimally treated subjects (MTPs), or PTPs, when BeneFIX was used for on-demand treatment of bleeding episodes, routine prophylaxis, and surgery. Most (89.1%) on-demand bleeding episodes were resolved with 1 or 2 infusions of BeneFIX. This favorable outcome was not restricted to any one bleed location, as 87.5% of joint bleeds and 88.6% of soft tissue/muscle bleeds resolved with one or 2 BeneFIX infusions. Most first infusions to treat a bleed were rated Excellent or Good (88.3%). These high ratings were associated with bleeding episodes occurring at each location site; the initial infusions used to treat the majority of joint (81.3%), soft tissue/muscle (88.6%), and multisite (100.0%) bleeding episodes were rated Excellent or Good.

The majority of bleeding episodes (61.4%) occurred >48 hours after the last BeneFIX dose and one subject raised his prophylaxis dose regimen (from 42 IU/kg/week to 55 IU/kg/week). Four (4) subjects had their on-demand regimen changed to routine prophylaxis. All 76 (100%) investigator assessments of overall response in subjects treated with BeneFIX were rated as Very Useful/Useful.

The most frequent treatment-emergent adverse events (TEAEs) reported in this study were fever and infection (14 [56%] each); rhinitis (12, 48%); cough increased (10, 40%); vomiting (9, 36%); accidental injury (8, 32%); rash (6, 24%); and abnormal laboratory tests, diarrhoea, conjunctivitis, and otitis media (3 [12%] each), which are not unexpected frequent events for this subject population. Related TEAEs were abnormal laboratory tests and rash (2 [8%] each); and allergic reaction, urticaria, FIX inhibition, local reaction to procedure, and increased cough (one [4%] each). The mild hematomas (one each in 2 subjects) were the only related treatment-emergent haemophilia events (TEHEs).

One of 25 subjects (4%) had one severe TEAE considered related to BeneFIX, FIX inhibition. No subjects had a severe TEHE considered related to BeneFIX. No life-threatening TEAEs or TEHEs considered related to BeneFIX were reported. In total, one

subject had one treatment-emergent serious adverse event (TESAE) (FIX inhibitor). There were no serious haemophilia events.

No deaths were reported in the study. No withdrawals due to adverse events (AEs) were reported in the study.

The development of FIX inhibitor is an event of interest; this subject also had allergic-type manifestations. There were no reports of thrombogenicity or red blood cell (RBC) agglutination in the syringe or tubing.

## 6.2 Study rationale

Regulatory authorities in Europe (EMA) increasingly request the provision of sufficient and valid data on post-marketing safety and efficacy of their products.[6] A European Registry on children below six years of age treated with BeneFIX like this non-interventional trial offers further useful data to support the safe and efficacious use of BeneFIX in children. It was conducted according to the recommendations for improving quality and transparency of non-interventional trials.

The primary objective was to collect safety data for BeneFIX in a cohort of subjects aged below 6 years. The data obtained in this age group will contribute to already available data.

This non-interventional study was designated as a Post-Authorisation Safety Study (PASS) and was conducted voluntarily by Pfizer Limited.

# 7 Research question and objectives

The objective of the study is to collect data in Europe regarding safety and efficacy of treatment with BeneFIX in children below 6 years of age treated in the routine clinical setting.

## 7.1 Primary Endpoint

- Safety data related to treatment of haemophilia B with BeneFIX in children below 6 years of age treated in the routine clinical setting.

## 7.2 Secondary Endpoint

- Efficacy data related to treatment of haemophilia B with BeneFIX in children below 6 years of age treated in the routine clinical setting.

# 8 Amendments and updates

| Number | Date | Section of study protocol | Amendment or update | Reason |
|---|---|---|---|---|
| 1 | 15 APR 2013 | Section 1.1 (PASS designation) | Include information for PASS study | Classification of the Study as a PASS |

# 9 Research methods

## 9.1 Study design

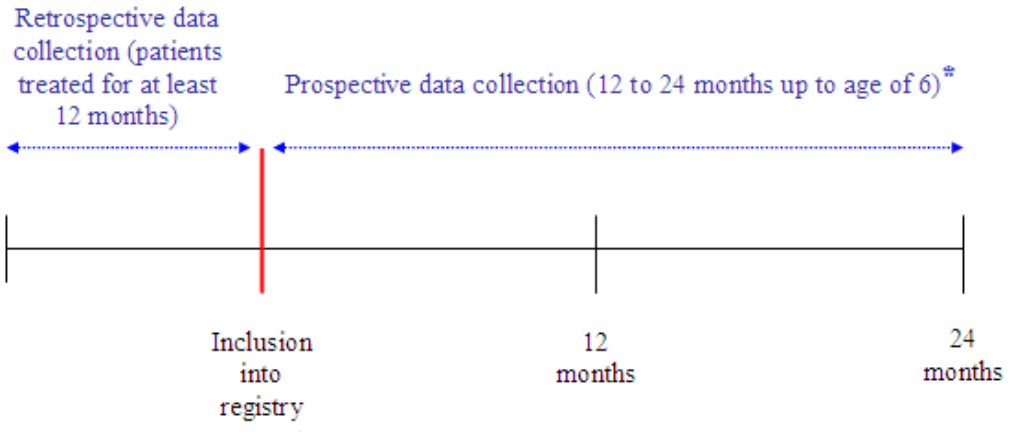
This was a two phase, non-interventional, multicentre registry study including a retrospective (Retrospective phase) and/or prospective (Prospective phase) data collection phase (Figure 1).

Retrospective data were collected only if subjects had been treated with BeneFIX for at least 12 consecutive months prior to their inclusion in the study. In order to ensure consistent data quality, retrospective documentations did not cover a time phase longer than 8 years ago, i.e. if a subject was 8 years of age, their treatment with BeneFIX between 0-6 years of age would still be retrospectively documented. Furthermore, subjects could be removed from the study if they were administered any other products for the treatment of Haemophilia B; for example, if a subject was treated with BeneFIX for 7 months, another product for 2 months, and then BeneFIX for another 13 months, then the subject would be considered to be included in the retrospective phase of data collection only for the 13 months. Prior BeneFIX treatment information would be collected only in the medical history.

Prospective data were collected if subjects were able to follow 12 to 24 months of treatment with BeneFIX before they reached 6 years of age.

Regarding the prospective phase, the data collection cut off was scheduled for 31st July 2016, for this reason it happened that for some subjects less than 12 months were accrued. The last visit performed before 31st July 2016, following clinical practice, was collected in electronic case report form (eCRF).

**Figure 1 Study Design**

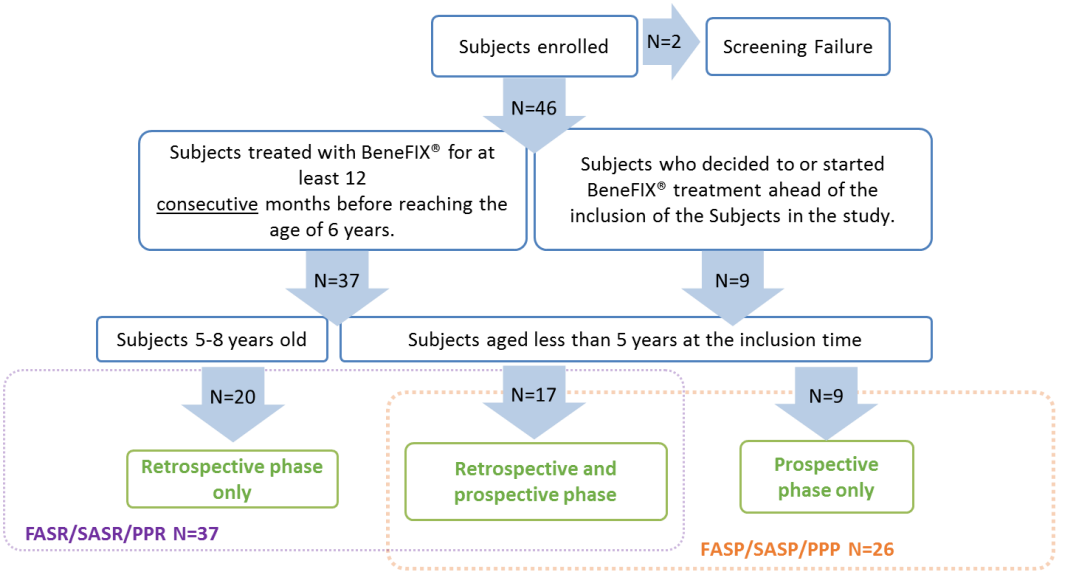



*All the visits performed following clinical practice by 31st July 2016, were collected in eCRF. Participating investigators were not influenced in their decision making and routine proceedings in any way.

Therefore, subjects could be enrolled in both phases of the study or in either the retrospective or prospective phase only, provided they met the collection criteria above

(Figure 2). Any subjects who did not meet the data collection criteria for the prospective phase were removed from the study at the end of the retrospective phase.

**Figure 2 Subject flow**




- Subjects also enrolled in the prospective phase (n=17)
- Subjects 6-8 year-old at the time of inclusion (n=14)
- Subjects 5 year-old at the time of inclusion- not able to accrue 12 months of prospective data (n=3)
- Subjects discontinued treatment before reaching 6 years of age (n=3)

- Completed 24 months of prospective observation (n=12)
- Reached the age of 6 years (n=1)
- Administrative cut off on 31st July 2016 (n=10)
- Insufficient Clinical Response (n=1)
- Subjects discontinued treatment before rich the 6 years of age or completed 24 months of prospective observation (n=2)

## 9.2 Setting

Approximately 50 subjects were planned for inclusion in the registry from approximately 25 sites across Italy, Spain, Sweden and United Kingdom.

All subjects enrolled had to meet the usual prescribing criteria for BeneFIX as per the local product information and had to be entered into the study at the investigator's discretion.

Subjects could be enrolled in both the retrospective and prospective part or in only one of the two, respectively.

Upon informed consent and when eligible for retrospective data collection, subject's charts were assessed retrospectively for at least 12 months on treatment with BeneFIX. If the subject received treatment with BeneFIX for more than 12 months, data from the entire time phase before reaching 6 years of age or inclusion in the study were collected retrospectively.

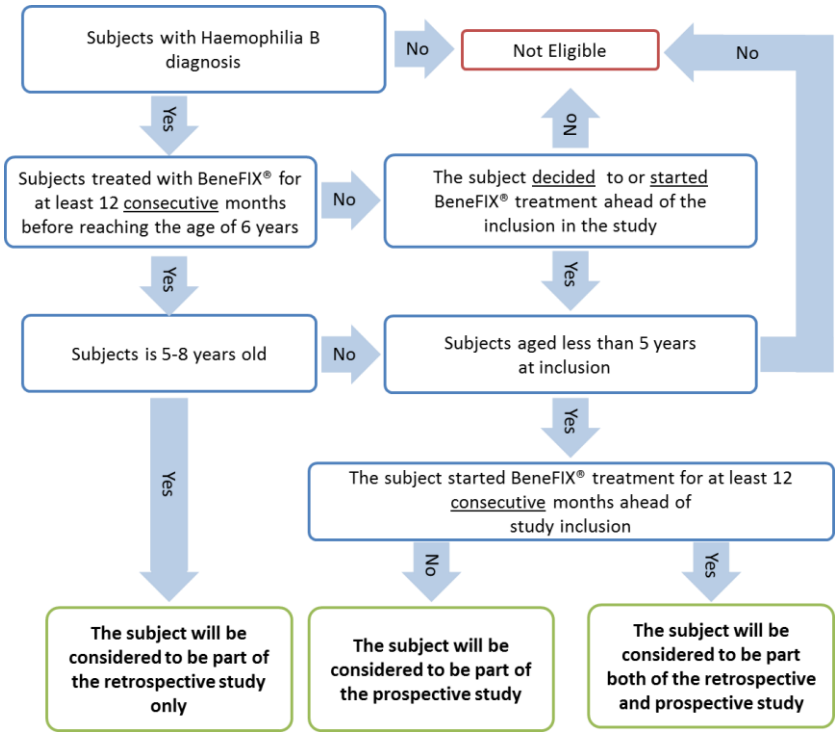
When eligible for prospective data collection, subjects' treatment outcomes were documented prospectively for up to 24 months or until the subject reached the age of 6 years. A minimum prospective collection phase of 12 months was attained before the subject reached the age of 6 years.

Regarding the prospective phase, the data collection cut off was scheduled for 31st July 2016, for this reason some subjects had less than 12 months data accrued. The last visit performed before 31st July 2016, following clinical practice, was collected in the eCRF.

The use and dosage for BeneFIX was based on the approved SmPC and was adjusted solely according to medical and therapeutic necessity. All treatment decisions followed the general clinical practice and were not influenced by this study protocol in any way.

**The study flow chart is shown in Figure 3.**

**Figure 3 Study Flow Chart**



## 9.3 Subjects

### 9.3.1 Selection criteria

Inclusion criteria

1) Subject has a diagnosis of Haemophilia B.

In addition, subjects had to meet one or both of the following criteria to be eligible for enrolment into the study:

2.1) Subjects treated with BeneFIX for at least 12 consecutive months before reaching the age of 6 years. These subjects were allowed to have a maximum age of 8 years at time of inclusion.

2.2) Subjects who were able to accrue at least 12 months in the prospective phase before reaching the age of 6 years. The treatment with BeneFIX had to have been in any case decided or started ahead of the inclusion of the subject in the study.

Regarding the prospective phase, the data collection cut off was scheduled for 31st July 2016, for this reason some subjects had less than 12 months data accrued. The last visit performed before 31st July 2016, following clinical practice, was collected in the eCRF.

Evidence of an informed consent document prior to any trial-related procedure was performed, signed and dated by the subjects' parents indicating that they (or a legally acceptable representative) had been informed of all pertinent aspects of the study.

Exclusion criteria

Subjects meeting the following criteria were not permitted to enter the study:

1) Subjects treated with a product for the treatment of haemophilia B other than BeneFIX over the retrospective and the prospective collection phase.

Subjects could withdraw from the study at any time their parents (or a legally acceptable representative) could request, or they could be withdrawn at any time at the discretion of the investigator or sponsor for safety, behavioural, or administrative reasons. In any circumstance, every effort could be made to document subject outcome, if possible. The investigator could inquire about the reason for withdrawal and follow-up with the subject regarding any unresolved AEs.

If the subject withdrew from the study, and also parents (or a legally acceptable representative) withdrew consent for disclosure of future information, no further evaluations could be performed, and no additional data could be collected. The sponsor could retain and continue to use any data collected before such withdrawal of consent.

## 9.4 Variables

### 9.4.1 Primary efficacy variable

Considering that safety was the primary endpoint of this study, all efficacy endpoints were considered secondary and were reported together in Section 9.4.2.

### 9.4.2 Secondary efficacy variable

Efficacy data in retrospective part and prospective part:

- Annualized bleeding rates (ABRs) for all bleeds and according to bleed location
- Responses to on-demand treatment with BeneFIX for bleeds of subjects on prophylactic or on-demand treatment regimens, respectively (4-point scale of assessment: excellent, good, moderate, no response)
- The incidence of less-than-expected therapeutic effect (LETE) was assessed by the investigator using the criteria listed in Section 9.8.1
- Lack of effect, defined as the failure of expected pharmacologic action or therapeutic benefit

Efficacy data in prospective part of the study only:

- Total number of days missed from work by parents/caregivers
- Total number of days when the subject was affected in daily activities due to his disease

### 9.4.3 Primary safety variables

- All AEs and Serious AEs (SAEs) related to BeneFIX during treatment (retrospective phase) – ADRs and SADRs
- All AEs and SAEs during treatment with BeneFIX (prospective phase) – TEAEs and TESAEs
- Events of special interest (ESIs)
  - Inhibitor development
  - Allergic reaction
  - Thrombotic event
  - RBC agglutination in tubing or syringe
  - Low recovery
- As bleeding events in haemophilia are part of the underlying disease they were not reported as adverse events unless they fulfil the definition of LETE

### 9.4.4 Other variables

- Demographic variables (date of birth, height, weight, ethnic group)
- Regular attendance of school
- Date of onset of the treatment with BeneFIX
- Haemophilia B previous therapy
- Disease severity (including genetic mutation if identified in routine clinical practice)

- Family history
- Disease history
- Dose of FIX at the beginning of the retrospective phase and/or prospective phase (treatment regimen, target value in IU/kg, amount of IU actually administered per infusion, frequency of administration)
- Any changes to the treatment regimen: dose and frequency of administration over the course of the retrospective phase and/or prospective observational phase and reasons for this change
- FIX activity and recovery if available and assessed in routine clinical visits
- Medicinal or non-medicinal concomitant therapy

## 9.5 Data sources and measurement

Data were collected from the subject's treatment records and from their treatment diaries. All data collected had been assessed in routine clinical practice. Due to the non-interventional nature of this study, no additional visits or procedures were requested for the study.

## 9.6 Bias

The voluntary participation of investigators constituted a selection bias observed for this type of study. Investigational sites were recruited within a representative list of the country's centres in terms of size, care management system and practices.

Subject's selection constituted another potential selection bias classically associated with non-interventional studies. Voluntary or involuntary selection of subjects in a study by investigators is inevitable, but this bias was limited by systematic attempts of the investigators to enrol subjects in the study.

The pragmatic nature of this study (which involved non-intervention on usual subject management practices) complicated the collection of follow-up data and may have increased the number of subjects lost to follow-up.

## 9.7 Study size

A statistical sample size calculation was not performed for this study since no statistical hypotheses were tested; thus, there was no need for determining statistical power. An approximation of 50 subjects was planned for inclusion in this study.

## 9.8 Data transformation

Once the eCRF was built, the clinical data manager (and other parties as appropriate) conducted User Acceptance Testing (UAT). The tester entered data into the eCRF and recorded whether it functioned as intended.

Most of the expected variables, derivations, range checks and consistency checks were built in the eCRF system, but some checks needed to be programmed separately for technical reasons.

Manual queries based on edit checks programmed and manual review listings were raised by the Clinical Data Manager in the data entry application.

Transfer of data for statistical analysis from the eCRF system to datasets was done using an export program created in SAS®.

All electronic data files delivered were password protected. Information about the password was sent separately to the Sponsor.

For the management of activities described in the Statistical Analysis Plan, the statistical software SAS 9.2 was used.

## 9.8.1 Definitions

### 9.8.1.1 Annualized bleeding rate (ABR)

The ABR or the annualized number of bleeding episodes was derived for each subject for each treatment phase (retrospective and prospective) by using the following formula:

ABR = number of bleeds during the treatment phase/ (number of days in treatment phase / 365.25)

Number of days in each treatment phase was defined as below:

– Retrospective Phase:

Number of days = Date of Informed Consent signature - Date of first BeneFIX administration recorded

– Prospective Phase:

Number of days = Date of last study visit in Prospective phase - Date of start of Prospective phase

The ABR was calculated for all bleeds and according to treatment regimen (on-demand or prophylaxis), type (spontaneous/traumatic) and bleed location (if available):

- Joint
- Soft tissue/ muscle
- Central Nervous System
- Other

### 9.8.1.2 Responses to the on-demand and prophylactic treatment with BeneFIX

The response to the on-demand and prophylactic treatment with BeneFIX, for all bleeds and according to bleeding location, was assessed by the subject/caregiver or investigator/ qualified staff by means of a 4-point scale (excellent, good, moderate, no response). One assessment per infusion was required.

In the event of a bleed in the on-demand setting (including those occurring during the prophylaxis phase), the 4-point response scale for an on-demand treatment of a bleeding episode with BeneFIX was defined as follows:

- Excellent: Definite pain relief and/or improvement in signs of bleeding starting within 8 hours after an infusion, with no additional infusion administered.

- Good:
    - Definite pain relief and/or improvement in signs of bleeding starting within 8 hours after an infusion, with at least one additional infusion administered for complete resolution of the bleeding episode.
    - Or definite pain relief and/or improvement in signs of bleeding starting after 8 hours following the infusion, with no additional infusion administered.
- Moderate: Probable or slight improvement starting after 8 hours following the infusion, with at least one additional infusion administered for complete resolution of the bleeding episode.
- No Response: No improvement at all between infusions or during the 24 hour interval following an infusion, or condition worsens.

#### 9.8.1.3 Incidence of less-than-expected therapeutic effect (LETE)

In this study, LETE could occur in 3 specific circumstances:

- Less than Expected Therapeutic Effect in the On-Demand Setting
- Less than Expected Therapeutic Effect in the Prophylaxis Setting
- Less than Expected Therapeutic Effect (Low Recovery)

These three types of LETEs, in aggregate and individually, were summarized as part of the efficacy information collected in this study.

The following criteria are the definitions for LETE in each type:

**a) Less than Expected Therapeutic Effect in the On-Demand Setting**

LETE occurred in the on-demand setting if 2 successive “No Response” ratings were recorded after 2 successive BeneFIX drug infusions, respectively (4-point response scale described in section above). The infusions had to be administered within 24 hours (≤24 hours) of each other for treatment of the same bleeding event in the absence of confounding factors (described below). Therefore, LETE in the on-demand setting was based on the response to treatment of a bleeding episode (including those occurring during the prophylaxis phase). The only confounding factors were as follows:

- Known presence or subsequent identification of a FIX inhibitor;
- Known inadequate dose for the type and/or severity of bleed in the opinion of the investigator;
- Delay of greater than 4 hours between onset of bleed to infusion;
- Delay of greater than 24 hours before administration of a follow-up infusion;
- Known compromised BeneFIX;
- Faulty administration of BeneFIX;
- The subject had an underlying, predisposing condition responsible for the bleed in the opinion of the investigator (e.g., kidney stones or use of medications known to impair platelet function, such as aspirin or NSAIDs).

Each LETE in the on-demand setting in the two phases was identified by investigating "No Response" and the BeneFIX drug infusion. The percentage of on-demand LETE for each subject was calculated as:

Percentage of LETE in On-demand Setting= number of bleeds identified as LETE/ the number of bleeding episodes treated in an on-demand setting during the treatment phase.

If data were available LETEs in on-demand setting were identified and percentage of LETEs was calculated for both the Interim Analysis and the Final Analysis.

**b) Less than Expected Therapeutic Effect in the Prophylaxis Setting**

LETE occurred in the prophylaxis setting if there was a spontaneous bleed within 48 hours (≤48 hours) after a regularly scheduled prophylactic dose of BeneFIX (which was not used to treat a bleed) in the absence of confounding factors. Therefore, LETE in the prophylaxis setting was the occurrence of a bleed. The only confounding factors were as follows:

- Known presence or subsequent identification of a FIX inhibitor;
- Known inadequate prophylactic dose (i.e., a dose less than that prescribed in subject's regimen);
- Known lack of adherence to the prescribed prophylaxis regimen;
- Bleed occurred in a target joint identified at the start of the study;
- Known compromised BeneFIX;
- Faulty administration of BeneFIX;
- The subject had an underlying, predisposing condition responsible for the bleed in the opinion of the investigator (e.g., kidney stones or use of medications known to impair platelet function, such as aspirin or NSAIDs);
- Traumatic injury responsible for bleeding.

Each LETE in the prophylaxis setting in the two phases was identified by investigating the bleeding within 48 hours of BeneFIX drug infusion. The percentage of prophylaxis LETE for each subject was calculated as:

Percentage of LETE in Prophylaxis Setting: number of prophylaxis infusions with a spontaneous bleed within 48 hours of the infusion/ number of bleeding incidences in prophylaxis setting during the treatment phase.

If data were available, LETEs in prophylaxis setting were identified and percentage of LETEs was calculated for both the Interim Analysis and the Final Analysis.

**c) Less than Expected Therapeutic Effect (Low Recovery)**

LETE could also be lower than expected recovery of FIX in the opinion of the investigator following infusion of BeneFIX in the absence of confounding factors. The only confounding factors for low recovery were as follows:

- Known presence or subsequent identification of a FIX inhibitor
- Known compromised BeneFIX
- Faulty administration of BeneFIX, including inadequate dosing.

Each reported occurrence of low recovery LETE was listed by treatment type (on-demand and prophylaxis).

#### 9.8.1.4 Lack of effect

The lack of effect was defined as the failure of expected pharmacologic action or therapeutic benefit. It was considered as a possible reason of treatment interruption. It was determined by the investigator assessment and recorded in the eCRF as an AE.

#### 9.8.1.5 FIX activity and recovery

FIX activity and recovery were recorded if available (i.e. assessed in routine clinical visits). The FIX activity and recovery were assessed at the local laboratory.

The incremental recovery, wherever possible, was defined as the IU/dL of FIX: C increase per IU/kg of study drug administered.

#### 9.8.1.6 Daily Living impact (Prospective part of the study only)

Daily Living impact variables were analysed if available (i.e. assessed in routine clinical visits).The following information was considered:

- Total number of days missed from work by parents/caregivers;
- Total number of days when the subject was affected in daily activities due to his disease.

The daily living impact data was collected 2-4 times a year and the total number of days in each category during the treatment phase (if available) was reported.

## 9.9 Statistical methods

### 9.9.1 Main summary measures

No statistical comparison was made between the two study phases (retrospective phase and prospective phase). Descriptive statistics (including number, mean, median, minimum, maximum, and standard deviation for continuous variables, or frequency and percentage (proportion) for binary or categorical variables) were reported for each study phase (retrospective phase and prospective phase), unless otherwise specified.

For proportions with a 95% confidence interval (95% CI) specified, the CI was 2-sided with an alpha level of 0.05.

### 9.9.2 Main statistical methods

The statistical software SAS® (version 9.2) was used to conduct the statistical analysis. The results were presented by using tables produced by programming using SAS.

#### 9.9.2.1 Demographic and other baseline characteristics

Subjects' continuous demographic and baseline variables were summarized using descriptive statistics (mean, standard deviation, median, minimum and maximum), while categorical variables were summarized using frequency tabulations (including disease parameters, and prior and concomitant therapies). No statistical hypothesis tests were performed on these characteristics.

The number and percentage of subjects enrolled for each study phase (retrospective and prospective) and the primary reason for discontinuation were displayed.

#### 9.9.2.2 Study drug and exposure to treatment

For BeneFIX, dosage statistics (mean, standard deviation median, minimum and maximum) were provided for:

– The number of infusions received by each subject

– The average interval (days) between infusions for each subject

– The actual dose received at the beginning of treatment (IU in both phases)

Duration of exposure was similarly reported together with a summary of the number of missed infusion per subjects who received prophylaxis treatment. Dosing summaries were provided by treatment regimen (on-demand and routine prophylaxis) for both the Interim Analysis and Final Analysis.

#### 9.9.2.3 Primary analysis

The primary objective of the study was safety assessment. Consequently the primary analysis is described in details at Section 9.9.2.5.

#### 9.9.2.4 Secondary analysis

Descriptive statistics were provided for each of the secondary endpoints described in Section 9.8.1. Statistical hypothesis tests were not conducted.

ABRs were descriptively summarized, including by bleed type (i.e. spontaneous or traumatic), by bleed location (i.e. joint, soft tissue/muscle, etc.), and by treatment type at the occurrence of bleeding (i.e. on-demand, prophylaxis).

The response to the treatment with BeneFIX (4-point scale: excellent, good, moderate, no response) was descriptively summarized for all bleeds (number and proportion) and according to bleeding location (i.e. joint, soft tissue/muscle, etc.). For each subject the assessment of all infusions was considered.

LETE was summarized in aggregate and individually according to specific setting of occurrence (On-Demand Setting, Prophylaxis Setting, and Low Recovery).

Lack of Effect was summarized in aggregate only.

#### 9.9.2.5 Adverse events

The safety analysis included all AEs, SAEs and Events of special interest (ESIs) occurred during treatment with BeneFIX (see Section 9.4.3). A prior Interim Analysis was planned on the safety analysis set for retrospective data population (SASR). However, this interim analysis was not finally performed due to the early administrative cut off of the study. Therefore, all the results of the retrospective and prospective phases are presented in the current final CSR. ESI for this study were: FIX inhibitor development, allergic reaction, thrombotic event, RBC agglutination in tubing or syringe, and Low recovery that were associated with LETE. ESIs were collected in the study eCRF in the same way of AEs but with specific extra information to characterize the type of ESI occurred.

AEs, SAEs and ESIs were summarized for each treatment phase using the events with the date of onset within that treatment phase. In case of an event occurred at the retrospective phase and increased the severity at the prospective phase, it was counted in the analysis of data for both phases.

AEs, SAEs and ESIs were coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 19.0.

The frequency of AEs, SAEs and ESIs were tabulated by MedDRA System Organ Class (SOC) and preferred term (PT). In the by-subject analysis, a subject having the same event more than once within a treatment phase was counted only once. AEs, SAEs and ESIs were summarized by worst severity reported within the treatment phase. AEs, SAEs and ESIs leading to death or to discontinuation from treatment, events classified as severity grade 3 or higher and study related events were listed separately for the retrospective and prospective phases.

Incidence density rate (IDR) for individual ESIs was derived for each phase as: ID = number of ESIs / sum of treatment time for each individual in the population.

IDR was provided together with two-sided 95% CIs (calculated from the binomial distribution). IDR was defined as the ratio of number of new ESIs in the calendar period over accrued population time (years).

Relevant summary tables were provided by country, that is, by pooling data from all centres within a specific country.

#### 9.9.2.6 Other assessments

The incremental recovery (FIX activity and recovery) recorded in the eCRF was summarized descriptively through appropriate data tabulations and descriptive statistics. Daily Living impact variables were analysed in the prospective part of the study and only if available (i.e. assessed in routine clinical visits).

Summary statistics (mean, standard deviation median, minimum and maximum) were provided for:

- Total number of days missed from work by parents/caregivers;
- Total number of days when the subject was affected in daily activities due to his disease.

### 9.9.3 Missing values

Missing data will not be imputed.

### 9.9.4 Sensitivity analyses

No sensitivity analyses were conducted.

### 9.9.5 Amendments to the statistical analysis plan

**There were several amendments made to the SAP, culminating in Version 2.0 (Table 2).Table 2 SAP Amendments**

| Version | Version | Author | Description |
|---|---|---|---|

| | **Date** | | |
|---|---|---|---|
| 18-Jun-2013 | 1.0 | Marco André Bassano | Initial release in according with suggestions from the RP4G group. |
| 10-Mar-2014 | 1.1 | Veronica Anna Pagano | Added list of tables and data summaries (section 9). Minor corrections & editorial changes. |
| 23-Oct-2015 | 1.2 | Veronica Anna Pagano | Updated document version history. |
| 22-Jan-2016 | 2.0 | Veronica Anna Pagano | Modified populations for analysis of data. Added details about Adverse events analysis. Minor editorial changes. |

#### 9.9.5.1 Changes in the Planned Analyses of the Study

During the preparation of this Post-Authorisation Safety Study report, several changes have been made to the analysis and reporting of both study phases:

1) In the retrospective phase, AEs were described as Adverse Drug Reactions (ADRs) as only AEs related to BeneFIX treatment were collected.
2) In the prospective phase, AEs were described as Treatment-Emergent AEs (TEAEs) as these could be related or unrelated to BeneFIX treatment.
3) The reference to data collection cut off (early study closure) was updated in relation to the early study closure notification sent to the principal and any additional investigators.
4) During the initial analysis (03 April 2017 – 18 December 2017) two subjects who were receiving an immune tolerance induction (ITI) regimen were included in the efficacy analysis of both phases. Tables were revised due to reporting that was inconsistent with protocol specifications.
5) During the initial analysis (03 April 2017 – 18 December 2017), four cases reporting FIX inhibitor were included in the tables reporting safety events (three in the retrospective period, one in the prospective period). However, these events occurred outside the protocol specified data collection reporting period and prior to consent and therein did not occur during the study but form part of the respective patients' medical history. Tables have been corrected accordingly and reflected in the CSR.

## 9.10 Quality control

**Monitoring**

The Sponsor or representative's monitor was responsible for verifying the eCRF at regular intervals throughout the study to verify the adherence to the protocol; completeness, accuracy, and consistency of the data; and adherence to local regulations on the conduct of clinical research. 100% of the data entered into the eCRF was verified against the source data at the site. Manual queries based on edit checks programmed and manual review listings were raised by the Clinical Data Manager in the data entry application.

Queries were resolved by the investigator or his authorized designee in the data entry application. The investigator was responsible for resolving and signing these queries.

**Access to source data and documentation**

The completed original eCRFs are the sole property of Pfizer and should not be made available in any form to third parties, except for authorized representatives of Pfizer or appropriate regulatory authorities, without written permission from Pfizer.

The investigator had ultimate responsibility for the collection and reporting of all clinical, safety and laboratory data entered on the eCRFs and any other data collection forms (source documents) and ensuring that they were accurate, authentic / original, attributable, complete, consistent, legible, timely (contemporaneous), enduring and available when was required. The eCRFs were signed by the investigator or by an authorized staff member to attest that the data contained on the eCRFs was true. After all data had been entered on the eCRF, data entry was closed by the user's electronic signature (user's login and password). Any corrections to entries made in the eCRFs, source documents were dated, initialled and explained (if was necessary) and did not obscure the original entry.

In most cases, the source documents were the hospitals or the physician's subject chart. In these cases data collected on the eCRFs matched the data in those charts. In haemophilia subjects, another source document was the subject diary from which data was collected in this study.

In some cases, the eCRF, or part of the eCRF, could also serve as source documents. In these cases, a document was available at the investigator's site as well as at Pfizer and clearly identified those data that were recorded in the eCRF, and for which the eCRF stood as the source document.

**Record retention**

To enable evaluations and/or audits from regulatory authorities or Pfizer, the investigator agreed to keep records, including the identity of all participating subjects (sufficient information to link records, e.g., eCRFs and hospital records), all original signed informed consent forms, copies of all eCRFs, serious adverse event forms, source documents, and detailed records of treatment disposition, and adequate documentation of relevant correspondence (e.g., letters, meeting minutes, telephone calls reports). The records were retained by the investigator according to local regulations, or as specified in the Clinical Study Agreement, whichever was longer.

If the investigator became unable for any reason to continue to retain study records for the required period (e.g., retirement, relocation), Pfizer was prospectively notified. The study records were transferred to a designee acceptable to Pfizer, such as another investigator, another institution, or to an independent third party arranged by Pfizer. The investigator obtained Pfizer's written permission before disposing of any records, even if retention requirements had been met.

**Quality assurance and audit**

To ensure compliance with relevant regulations, data generated by this study were available for inspection upon request by representative of the European Medicine Agency (EMA), and other national authorities and local health authorities where the study was conducted, the Sponsor and representatives, and the IRB/IEC for each study site. The investigator permitted authorized representatives of the sponsor, the respective national or local health authorities, and auditors to inspect facilities and records relevant to this study.

Auditors, IRB/IEC and/or regulatory inspectors also had access to the eCRFs and source documents.

# 10 Results

## 10.1 Retrospective phase

### 10.1.1 Participants

A total of 38 paediatric subjects were screened for inclusion in the retrospective phase of the EUREKIX study. Eligibility was defined as meeting inclusion criterion 1, at least one sub-criterion of inclusion criterion 2 (2.1 and 2.2), and not meeting the exclusion criterion. One (2.6%) subject was classified as a screening failure due to being nine years old at the time of enrolment. The remaining 37 subjects were included in the retrospective full analysis set (FASR); safety analysis set (SASR), and per protocol analysis set (PPR) populations (Table 3).

All subjects met inclusion criterion 1, 37 (97.4%) subjects met inclusion sub-criterion 2.1, and 17 (44.7%) subjects met sub-criterion 2.2 (Annex 1.2: Table 14.1.3). No subject met the exclusion criterion (Annex 1.2: Table 14.1.4).

**Table 3 Retrospective study population**

| | | ***Retrospective Phase*** |
|---|---|---|
| FASR | No | 1 (2.63%) |
| | Yes | 37 (97.37%) |
| SASR | No | 1 (2.63%) |
| | Yes | 37 (97.37%) |
| PPR | No | 1 (2.63%) |
| | Yes | 37 (97.37%) |

Full Analysis Set for Retrospective (FASR), Safety analysis set for Retrospective data (SASR), Per Protocol analysis sets for Retrospective data (PPR)

*Source: Table 14.1.2*

Fourteen (36.8%) subjects were screened in Italy, ten (26.3%) were screened in Spain, eight (21.1%) were screened in Sweden and the remaining six (15.8%) subjects were screened from the UK, respectively (Table 4). The mean (SD) time treated with BeneFIX was 3.4 (1.4) years (Table 4). Upon completion of the retrospective phase, 17 (46.0%) subjects continued to the prospective phase. The remaining 20 (54.1%) subjects stopped the study at the end of the retrospective phase as they were not eligible for continuation into the prospective phase. Fourteen (37.8%) subjects were ineligible for continuation as they were six years old by the end of the retrospective data collection phase; three (8.1%) subjects would reach the age of six years old prior to the end of the prospective data collection phase; and three (8.1%) subjects discontinued treatment (two subjects switched treatment and one subject took part in a clinical trial).

## Table 4 Retrospective phase subject disposition

| | | ***Retrospective Phase*** |
|---|---|---|
| Country | Total | 38 (100.00%) |
| | IT | 14 (36.84%) |
| | SP | 10 (26.32%) |
| | SW | 8 (21.05%) |
| | UK | 6 (15.79%) |
| Screening Failure | Total | 38 (100.00%) |
| | No | 37 (97.37%) |
| | Yes | 1 (2.63%) |
| Reason to stop the retrospective period | Total | 37 (100.00%) |
| | Subject 5 years old - not able to accrue 12 months prospective data | 3 (8.11%) |
| | Subject discontinued treatment* | 3 (8.11%) |
| | Subject enrolled also in the phase B | 17 (45.95%) |
| | Subject has reached the age of 6 years | 14 (37.84%) |
| Time in retrospective phase (years) | n | 37 |
| | N. missing | 0 |
| | Mean (95%CI) | 3.37 (2.88 , 3.85) |
| | Standard deviation (SD) | 1.44 |
| | Median | 3.15 |
| | (Q1, Q3) | (2.02, 4.64) |
| | (Min, Max) | (1.03, 6.00) |

(*) 2 subjects discontinued treatment for long-acting product, 1 subject took part in a clinical trial
SF: Subject 1027-001 included into the respective phase of the study with the age of 9 years

*Source: Table 14.1.1*

## 10.1.2 Descriptive Data

All 37 subjects were male, predominantly Caucasian (91.9%), and had a mean (SD) age of 5.0 (2.1) years old (Table 5). Most subjects (n=30; 81.1%) were attending school/kindergarten at the time of study inclusion.

**Table 5 Full analysis set for retrospective (FASR) phase: demographic data**

| | | ***FASR (N=37)*** |
|---|---|---|
| Ethnicity | | |
| | Caucasian | 34 (91.89%) |
| | Black | 2 (5.41%) |
| | Unknown | 1 (2.70%) |
| Age | n | 37 |
| | N. missing | 0 |
| | Mean (95%CI) | 5.04 (4.33 , 5.75) |
| | Standard deviation (SD) | 2.12 |
| | Median | 5.09 |
| | (Q1, Q3) | (3.15, 6.65) |
| | (Min, Max) | (1.96, 8.72) |
| Gender | | |
| | Male | 37 (100.00%) |
| School or kindergarten | | |
| | Yes | 30 (81.08%) |
| | No | 4 (10.81%) |
| | NA | 3 (8.11%) |

*Source: Table 14.1.9*

The mean (SD) age at which the subjects were diagnosed with haemophilia B was 0.5 (0.7) years old (approximately 6 months old) and 25 (67.6%) had a family history of haemophilia B (Table 6). The mean age of the first bleed was 0.8 (0.8) years old and the first exposure to BeneFIX was 1.1 (0.8) years old.

Classification of haemophilia B by severity shows the majority of subjects had moderate (n=25; 67.6%) to severe (n=8; 21.6%) disease severity. The remaining 4 subjects (10.8%) presented mild haemophilia B. Furthermore, 21 (56.8%) subjects were reported to have experienced at least one haemarthrosis. In general, the first haemarthrosis was experienced at 2.1 (1.4) years old, roughly one year after the first exposure to BeneFIX. Joints were not targeted for any subject, and 28 (75.7%) subjects were reported to have a factor IX gene mutation – the most common were missense mutations which were reported for 14 (37.8%) subjects (Annex 1.2: Table 14.1.10).

## Table 6 FASR disease parameters

| | | | ***FASR (N=37)*** |
|---|---|---|---|
| Age of diagnosis (years) | n | | 37 |
| | N. missing | | 0 |
| | Mean (95%CI) | | 0.49 (0.26 , 0.71) |
| | Standard deviation (SD) | | 0.68 |
| | Median | | 0.36 |
| | (Q1, Q3) | | (0.00, 0.78) |
| | (Min, Max) | | (0.00, 3.78) |
| Age of first bleed (years) | n | | 36 |
| | N. missing | | 1 |
| | Mean (95%CI) | | 0.82 (0.56 , 1.09) |
| | Standard deviation (SD) | | 0.78 |
| | Median | | 0.72 |
| | (Q1, Q3) | | (0.34, 1.00) |
| | (Min, Max) | | (0.00, 3.78) |
| Age at first exposure to BeneFIX (years) | n | | 37 |
| | N. missing | | 0 |
| | Mean (95%CI) | | 1.06 (0.80 , 1.32) |
| | Standard deviation (SD) | | 0.78 |
| | Median | | 0.90 |
| | (Q1, Q3) | | (0.68, 1.21) |
| | (Min, Max) | | (0.01, 3.78) |
| Family history | | | 37 (100.00%) |
| | Yes | | 25 (67.57%) |
| | No | | 11 (29.73%) |
| | NA | | 1 (2.70%) |
| Severity of Haemophilia B | | | 37 (100.00%) |
| | Severe [FIX: C<1 IU/dL (%)] | | 25 (67.57%) |
| | Moderate [FIX: C 1 to 5 IU/dL (%)] | | 8 (21.62%) |
| | Mild [FIX: C >5 to 40 IU/dL (%)] | | 4 (10.81%) |
| Subjects with joint bleed | | | 37 (100.00%) |
| | Yes | | 21 (56.76%) |
| | No | | 16 (43.24%) |
| | Age at first joint bleed | n | 21 |
| | | N. missing | 0 |
| | | Mean (95%CI) | 2.07 (1.46 , 2.68) |
| | | Standard deviation (SD) | 1.35 |
| | | Median | 1.70 |
| | | (Q1, Q3) | (0.99, 2.44) |
| | | (Min, Max) | (0.59, 5.45) |
| Target joints | | | 37 (100.00%) |
| | No | | 37 (100.00%) |
| Mutation | | | 37 (100.00%) |
| | Yes | | 28 (75.68%) |
| | No | | 9 (24.32%) |

*Source: Table 14.1.10*

For inclusion in this PASS, subjects were required to be treated with BeneFIX during the retrospective study phase; however, they may have received another product prior to study participation. BeneFIX was the first treatment for haemophilia B for 32 (86.5%) subjects, with the remaining five (13.5%) subjects having received another product as the first treatment for haemophilia B. In total, seven (18.9%) subjects had received another treatment (before or after the first BeneFIX administration): tranexamic acid (n=3; 8.1%), FIX (n=2; 5.4%), and plasma (n=2; 5.4%) [Table 7].

**Table 7 FASR haemophilia B prior therapy**

| | | | ***FASR (N=37)*** |
|---|---|---|---|
| Did the subject receive BeneFIX as first treatment for Haemophilia B? | | | 37 (100.00%) |
| | Yes | | 32 (86.49%) |
| | No | | 5 (13.51%) |
| Did the subject receive any other product than BeneFIX for treatment of Haemophilia B? | | | 37 (100.00%) |
| | No | | 30 (81.08%) |
| | Yes | | 7 (18.92%) |
| | Preferred Name (*) | | |
| | | TRANEXAMIC ACID | 3 (42.86%) |
| | | FIX | 2 (28.57%) |
| | | PLASMA | 2 (28.57%) |
| | | EPTACOG ALFA (ACTIVATED) | 1 (14.29%) |
| | | FACTOR II (PROTHROMBIN) | 1 (14.29%) |
| | | RED BLOOD CELLS | 1 (14.29%) |

(*) Percentage calculated on the total of subjects who received other product
Previous therapy for Haemophilia B coded using WHO-DDE version 16.1.

*Source: Table 14.1.11*

Furthermore, a history of inhibitors or allergic reactions to FIX products was low in this population. Inhibitors against FIX were recorded for two (5.4%) subjects and were considered related to BeneFIX treatment (Listing 16.2.6.2). One inhibitor event was of mild severity and one was of severe severity; both patients temporarily stopped BeneFIX for the treatment of the inhibitors before reinitiating treatment with an ITI regimen. Allergic reactions against FIX were documented for four (10.8%) subjects (Table 8). Of the four subjects with allergic reactions, three were not related (two cases of sleepiness and one case of hives and generalised urticaria) and one was related (anaphylaxis) to BeneFIX treatment. Lastly, there were no documented cases of RBC agglutination in this population.

**Table 8 FASR disease history (before study inclusion)**

| | | | ***FASR (N=37)*** |
|---|---|---|---|
| History of inhibitors | | | 37 (100.00%) |
| | Yes | | 2 (5.41%) |
| | No | | 35 (94.59%) |
| History of allergic reactions | | | 37 (100.00%) |
| | Yes | | 4 (10.81%) |
| | No | | 33 (89.19%) |
| | Signs or symptoms that resulted in diagnosis of allergic reactions(*) | | 4 (100.00%) |
| | | Hives | 1 (25.00%) |
| | | Generalized Urticaria | 1 (25.00%) |
| | | Anaphylaxis | 1 (25.00%) |
| | | Sleepiness | 2 (50.00%) |
| RBC agglutination | | | 37 (100.00%) |
| | No | | 34 (91.89%) |
| | NA | | 3 (8.11%) |

2 subjects have a history of inhibitor against BeneFIX products
[#1023-3 - Titres: 1.6; Peak: 2.2; Method of assessment: Bethesda; Lower detection value: 0.4]
[#1031-2 - Titres: 4.5; Peak: 4.5; Method of assesment: Bethesda; Lower detection value: 0.5].
(*) Not mutually exclusive categories

*Source: Table 14.1.12*

Relevant medical history was collected for all subjects and 15 (40.5%) subjects had a significant medical history (Annex 1.2: Table 14.1.13). No subject had prior hepatitis A, B, or C viral infections; similarly, there were no subjects with HIV. For immunisations, 22 (59.5%) subjects were immunized against hepatitis A and B. Within the FASR, there were 33 diseases/conditions, with the most common (surgical and medical procedures) being recorded in seven (18.9%) subjects. In terms of prior or concomitant medication, there was a high degree of variability – with the majority of treatments being given to one or two subjects (Annex 1.2: Table 14.1.14). The most common prior and concomitant medications were tranexamic acid (n=26; 70.3%) and paracetamol (n=13; 35.1%).

## 10.1.3 Efficacy Results

### 10.1.3.1 Annualised bleeding rates (ABR)

The ABR, or the annualized number of bleeding episodes, was derived for each subject for each treatment phase.

During the retrospective phase of the study, the mean (95% CI) bleeding rate in the 35 evaluated subjects was 3.4 (2.5, 4.4) times per year (Table 9).

Subjects were categorised as having a "prophylaxis" or "on-demand" regimen if they were receiving the regimen for more than 50% of the study period. At the beginning of the observational period, 34 (91.9%) subjects were receiving an on-demand regimen, two (5.4%) were receiving an ITI regimen, and one (2.7%) was receiving a prophylaxis regimen (Annex 1.2: Table 14.3.3.1). In the retrospective phase a total of 24 (64.9%) subjects received an overall prophylaxis regimen, 11 (29.7%) received an on-demand regimen, and two (5.4%) received an ITI regimen. The two subjects receiving an ITI regimen were excluded from all efficacy analysis. The mean (95% CI) ABR for the subjects

receiving a prophylaxis regimen was 3.7 (2.7, 4.7). The mean (95% CI) ABR for the subjects receiving an on-demand regimen was 3.0 (0.8, 5.1). In all 35 subjects, the mean (95% CI) ABR related to traumatic bleeds was 2.2 (1.5, 2.9). The mean (95% CI) ABR related to spontaneous bleeds was 0.5 (0.3, 0.7). For soft tissue/muscle bleeds and joint bleeds the mean (95% CI) ABR were 1.1 (0.6, 1.6) and 0.6 (0.4, 0.8), respectively. The mean (95% CI) ABR for central nervous system (CNS) bleeds was 0.1 (0.0, 0.1).

**Table 9 FASR annualised bleeding rates**

| | | ***FASR (N=37)*** |
|---|---|---|
| ABR (all bleeds) | n | 35 |
| | N. missing | 0 |
| | Mean (95%CI) | 3.44 (2.53 , 4.35) |
| | Standard deviation (SD) | 2.64 |
| | Median | 2.48 |
| | (Q1, Q3) | (1.89, 5.39) |
| | (Min, Max) | (0.00,11.39) |
| ABR (prophylaxis regimen) | n | 24 |
| | N. missing | 0 |
| | Mean (95%CI) | 3.67 (2.65 , 4.69) |
| | Standard deviation (SD) | 2.41 |
| | Median | 3.77 |
| | (Q1, Q3) | (1.93, 5.50) |
| | (Min, Max) | (0.00, 8.10) |
| ABR (on-demand regimen) | n | 11 |
| | N. missing | 0 |
| | Mean (95%CI) | 2.95 (0.84 , 5.06) |
| | Standard deviation (SD) | 3.14 |
| | Median | 2.01 |
| | (Q1, Q3) | (1.57, 2.68) |
| | (Min, Max) | (0.00,11.39) |
| ABR (spontaneous bleeds) | n | 35 |
| | N. missing | 0 |
| | Mean (95%CI) | 0.48 (0.28 , 0.68) |
| | Standard deviation (SD) | 0.58 |
| | Median | 0.28 |
| | (Q1, Q3) | (0.00, 0.94) |
| | (Min, Max) | (0.00, 1.94) |
| ABR (traumatic bleeds) | n | 35 |
| | N. missing | 0 |
| | Mean (95%CI) | 2.21 (1.53 , 2.89) |
| | Standard deviation (SD) | 1.98 |
| | Median | 1.40 |
| | (Q1, Q3) | (0.83, 3.18) |
| | (Min, Max) | (0.00, 7.45) |
| ABR (joint bleeds) | n | 35 |
| | N. missing | 0 |
| | Mean (95%CI) | 0.60 (0.37 , 0.84) |
| | Standard deviation (SD) | 0.69 |
| | Median | 0.37 |

| | | ***FASR (N=37)*** |
|---|---|---|
| | (Q1, Q3) | (0.00, 0.97) |
| | (Min, Max) | (0.00, 2.81) |
| ABR (soft tissue/muscle bleeds) | n | 35 |
| | N. missing | 0 |
| | Mean (95%CI) | 1.08 (0.60 , 1.56) |
| | Standard deviation (SD) | 1.40 |
| | Median | 0.60 |
| | (Q1, Q3) | (0.00, 1.68) |
| | (Min, Max) | (0.00, 6.41) |
| ABR (CNS bleeds) | n | 35 |
| | N. missing | 0 |
| | Mean (95%CI) | 0.06 ( -0.01 , 0.13) |
| | Standard deviation (SD) | 0.20 |
| | Median | 0.00 |
| | (Q1, Q3) | (0.00, 0.00) |
| | (Min, Max) | (0.00, 0.96) |
| ABR (Other bleeds) | n | 35 |
| | N. missing | 0 |
| | Mean (95%CI) | 1.65 (0.82 , 2.48) |
| | Standard deviation (SD) | 2.41 |
| | Median | 0.80 |
| | (Q1, Q3) | (0.00, 2.10) |
| | (Min, Max) | (0.00,11.39) |

Subjects are classified into treatment groups (On-demand or Prophylaxis) based on the length of time on each regimen during the study phase:
On-demand, <50% of the treatment phase on prophylaxis; Prophylaxis, >= 50% of the treatment phase on prophylaxis. Three subjects have been treated with ITI after developing inhibitor (they have been classified in a separate category).
The subjects #1031-2 and #1023-3, who received an ITI regimen during the study period due to a history of BENEFIX inhibitors, are excluded from this table.

*Source: Table 14.2.1.1*

### 10.1.3.2 Response to on-demand and prophylactic BeneFIX treatment

Response to on-demand treatment of bleeds reported by subjects receiving on-demand or prophylactic treatment regimens was assessed by a 4-point scale of ‘excellent’, ‘good’, ‘moderate’, or ‘no response’. An *Excellent* response was defined as a definite pain relief and/or improvement in signs of bleeding starting within 8 hours after an infusion, with no additional infusion administered. A *Good* response was defined as definite pain relief and/or improvement in signs of bleeding starting within 8 hours after an infusion (with at least one additional infusion administered for complete resolution of the bleeding episode), or definite pain relief and/or improvement in signs of bleeding starting after 8 hours following the infusion (with no additional infusion administered). A *Moderate* response was a probable or slight improvement starting after 8 hours following the infusion, with at least one additional infusion administered for complete resolution of the bleeding episode. Finally, *No Response* was defined as no improvement at all between infusions or during the 24 hour interval following an infusion, or condition worsens.

The overall highest response was *Excellent* for 33 (94.2%) subjects, with one (2.9%) exhibiting *Good* response and one (2.9%) ‘not-applicable’ response (Table 10). When

stratifying by treatment regimen, on-demand treatment with BeneFIX for bleeds for subjects prescribed either a prophylaxis or on-demand regimen had the majority of collected responses rated as *Excellent*; 23 out of 24 (95.8%) and 10 out of 11 (90.9%) subjects, respectively.

**Table 10 FASR response to BeneFIX treatment**

| | | ***FASR (N=37)*** |
|---|---|---|
| Best Response (Overall) | | 35 (100.00%) |
| | Excellent (1) | 33 (94.29%) |
| | Good (2) | 1 (2.86%) |
| | Moderate (3) | 0 (0.00%) |
| | No response (4) | 0 (0.00%) |
| | Not applicable (5) | 1 (2.86%) |
| | Not collected | 0 (0.00%) |
| Best Response (Prophylaxis regimen) | | 24 (100.00%) |
| | Excellent (1) | 23 (95.83%) |
| | Good (2) | 1 (4.17%) |
| | Moderate (3) | 0 (0.00%) |
| | No response (4) | 0 (0.00%) |
| | Not applicable (5) | 0 (0.00%) |
| | Not collected | 0 (0.00%) |
| Best Response (On-demand regimen) | | 11 (100.00%) |
| | Excellent (1) | 10 (90.91%) |
| | Good (2) | 0 (0.00%) |
| | Moderate (3) | 0 (0.00%) |
| | No response (4) | 0 (0.00%) |
| | Not applicable (5) | 1 (9.09%) |
| | Not collected | 0 (0.00%) |

(1) Definite pain relief and/or improvement in signs of bleeding starting within 8 hours after an infusion with no additional infusion administered
(2) Definite pain relief and/or improvement in signs of bleeding starting within 8 hours after an infusion with at leat one additional infusion administered for complete resolution of bleeding episode. Or definite pain relief and/or improvement in signs of bleeding starting after 8 hours following the infusion with no additional infusion administered
(3) Probable or slight improvement starting after 8 hours following the infusion with at least one additional infusion administered for complete resolution of the bleeding episode
(4) No improvement at all between infusions or during the 24 hour interval following an infusion or condition worsens
Subjects are classified into treatment groups (On-demand or Prophylaxis) based on the length of time on each regimen during the study phase: On-demand, <50% of the treatment phase on prophylaxis; Prophylaxis, >= 50% of the treatment phase on prophylaxis. Three subjects have been treated with ITI after developing inhibitor (they have been classified in a separate category).
The subjects #1031-2 and #1023-3, who received an ITI regimen during the study period due to a history of BENEFIX inhibitors, are excluded from this table.

*Source: Table 14.2.1.2*

### 10.1.3.3 Incidence of less-than-expected therapeutic effect (LETE)

A LETE occurred in the on-demand setting if two successive ‘no response’ ratings were recorded after two successive BeneFIX drug infusions. By contrast, a LETE occurred in the prophylaxis setting if there was a spontaneous bleed within 48 hours after a regularly scheduled prophylactic dose of BeneFIX (in the absence of confounding factors).

One LETE, a spontaneous bleed, was recorded and related to prophylaxis treatment (Annex 1.2: Table 14.2.1.3)

### 10.1.3.4 FIX activity and recovery

One International Unit (IU) of FIX activity is equivalent to that quantity of FIX in one ml of normal human plasma. Estimation of the required dose of BeneFIX can be based on the finding that one unit of FIX activity per kg body weight is expected to increase the circulating level of FIX, an average of 0.8 IU/dl (range from 0.4 to 1.4 IU/dl) in adolescents and adults.

In the retrospective phase, FIX activity (FIX:C) and recovery laboratory tests were recorded for 9 and 20 subjects, respectively (Table 11). Mean (95% CI) FIX:C was 26.0 (5.2, 46.7) IU/dL and the mean FIX:C recovery (as measured by an IU/dL of FIX:C increase per IU/kg of BeneFIX) was 1.4 (0.0, 3.0) IU/dL of FIX:C increase per IU/kg of BeneFIX.

**Table 11 FASR FIX activity and recovery**

| | | ***FASR (N=37)*** |
|---|---|---|
| Fix Activity (%) | n | 26 |
| | N. missing | 9 |
| | Mean (95%CI) | 13.51 (5.30 ,21.71) |
| | Standard deviation (SD) | 20.32 |
| | Median | 8.36 |
| | (Q1, Q3) | (2.75,14.81) |
| | (Min, Max) | (1.39,98.00) |
| Fix Activity (IU/dL) | n | 9 |
| | N. missing | 26 |
| | Mean (95%CI) | 25.97 (5.22 ,46.72) |
| | Standard deviation (SD) | 26.99 |
| | Median | 20.63 |
| | (Q1, Q3) | (2.00,45.48) |
| | (Min, Max) | (0.60,74.16) |
| Recovery | n | 20 |
| | N. missing | 15 |
| | Mean (95%CI) | 1.38 ( -0.24 , 3.01) |
| | Standard deviation (SD) | 3.47 |
| | Median | 0.70 |
| | (Q1, Q3) | (0.20, 0.86) |
| | (Min, Max) | (0.01,16.00) |

The subjects #1031-2 and #1023-3
who received an ITI regimen during the study period due to a history of BENEFIX inhibitors are excluded from this table.

*Source: Table 14.2.2.1*

## 10.1.4 Safety results

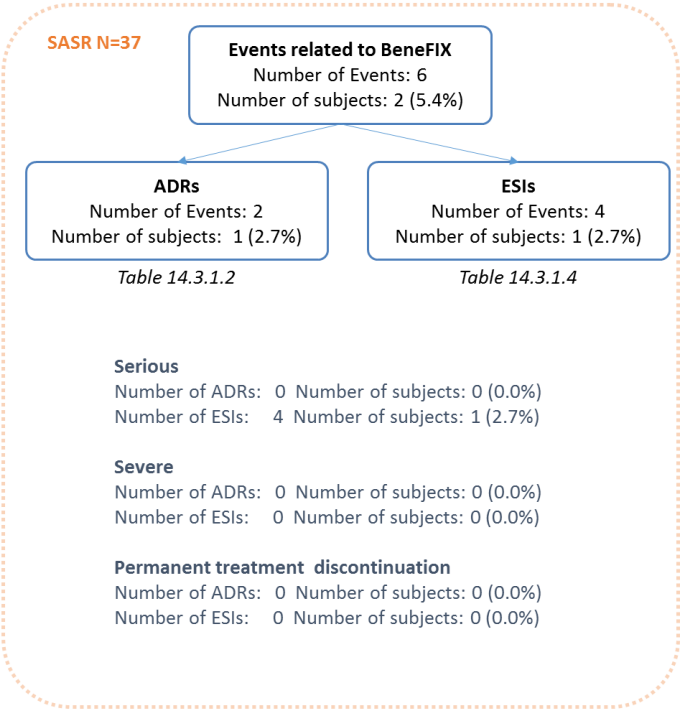
### 10.1.4.1 Brief summary of safety results

In the retrospective phase of the study two ADRs (ecchymosis and skin haemorrhage) were recorded in 1/37 (2.7%) subject based on the safety analysis population. Both of these ADRs were of mild severity and were resolved by the end of the study period (Table 12 and Annex 1.2: Tables 14.3.1.1, 14.3.1.2, & 14.3.1.9). None of them was considered as serious ADRs.

There were four ESIs experienced by one subject (2.7%) (Annex 1.2: Table 14.3.1.3 & 14.3.1.4); all considered to be serious (SESIs) (Annex 1.2: Table 14.3.2.1). The subject experienced hypersensitivity of mild severity on four occasions; No action with the treatment was taken and all ESIs were resolved by the end of the study phase.

No deaths were reported in this retrospective phase (Annex 1.2: Table 14.3.2.2). No subject experienced an ADR or ESI that led to permanent treatment discontinuation (Annex 1.2: Table 14.3.2.3). The individual lists of ADRs and ESI are provided in Annex 1.3: Listing 16.2.15 & 16.2.17.

**Figure 4 SASR summary of events**




### 10.1.4.2 Distribution of ADRs by SOC and PT

In the retrospective phase of this study the only ADRs encountered were within the Skin and subcutaneous tissue disorders SOC (PT Ecchymosis (n=1, 2.7%) and PT Skin haemorrhage (n=1, 2.7%)) (Table 13). Both were of mild severity and were resolved during the study period (Annex 1.2: Table 14.3.1.9); neither were considered to be a serious ADR.

**Table 12 SASR ADRs distributed by SOC and PT**

| SOC | PT | *SASR (N=37)* Mild |
|---|---|---|
| Number of subjects with at least one Treatment Emergent Adverse Event | | 1 |
| Skin and subcutaneous tissue disorders | Number of TEAEs | 2 |
| | Number of subjects | 1 (2.70%) |
| | Ecchymosis | 1 (2.70%) |
| | Skin haemorrhage | 1 (2.70%) |

TEAEs = Number of treatment emergent adverse events
Subjects who experienced multiple TEAEs within the same SOC and PT were counted only once, using the most severe rating
TEAEs coded with MedDRA Dictionary version 19.0
This table includes only events that began during the study period

*Source: Table 14.3.1.5*

### 10.1.4.3 Serious ADRs

None of the ADRs reported was considered as a serious ADR.

### 10.1.4.4 Events of special interest

In the retrospective phase, there were four ESIs in one (2.7%) subject. All ESIs reported were considered to be serious (SESIs) and are described in the next section (Table 13). The IDR for individual ESIs was calculated as the number of ESIs divided by the sum of treatment time for each individual in the population. The IDR (95% CI) for the individual ESIs in the retrospective phase was 0.032 (0.01, 0.08) (Annex 1.2: Table 14.3.1.11).

### 10.1.4.5 Serious events of special interest

Subject #1023-3 experienced four Hypersensitivity SESIs; all were mild in severity, required no action with the treatment, and were resolved by the end of the study (Table 13).

## Table 13 SASR SESIs

| SITE ID | PAT ID | Gender | Age | ESI | Term | SOC | PT | Onset | Resolved | Severity | Related | Action on Treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1023 | 3 | Male | 3.1 | Yes | ALLERGIC REACTION | Immune system disorders | Hypersensitivity | 18APR2013 | 19APR2013 | Mild | Yes | No action taken |
| | | | | Yes | ALLERGIC REACTION | Immune system disorders | Hypersensitivity | 01OCT2012 | 01OCT2012 | Mild | Yes | No action taken |
| | | | | Yes | ALLERGIC REACTION | Immune system disorders | Hypersensitivity | 30APR2013 | 30APR2013 | Mild | Yes | No action taken |
| | | | | Yes | ALLERGIC REACTION AFTER INJ BENEFIX | Immune system disorders | Hypersensitivity | 14APR2013 | 14APR2013 | Mild | Yes | No action taken |

This table includes only events that began during the study period

*Source: Table 14.3.2.1*

### 10.1.4.6 Lack of effect

In the retrospective phase there were three cases of a lack of effect documented in two (5.4%) subjects (Table 14). In all cases, the lack of effect was of mild severity, with one subject experiencing a lack of effect that was not related to BeneFIX, whereas the remaining subject experienced two events of lack of effect that were related to treatment. In all three cases, the dose of BeneFIX was increased and no action on the subject was required.

**Table 14 FASR lack of effect**

| Subject ID | SUBJID | Onset | Resolved | Severity | Related | Action on Treatment | Action on subject |
|---|---|---|---|---|---|---|---|
| 1023 | 4 | 15/03/2009 | 22/06/2009 | Mild | No | Dose Increased | No Action |
| 1051 | 1 | 02/03/2011 | UNK | Mild | Yes | Dose Increased | No Action |
| | 1 | 13/05/2013 | UNK | Mild | Yes | Dose Increased | No Action |

*Source: Table 14.2.1.4*

## 10.2 Prospective Phase

### 10.2.1 Participants

For the prospective phase of the study, a total of 27 subjects were eligible for inclusion, with one (3.7%) subject classified as a screening failure due to being older than five years old at the time of inclusion. Seventeen subjects who were enrolled in the retrospective phase of the study continued into the prospective phase. The remaining 26 (96.3%) subjects were included in the prospective full analysis set (FASP); safety analysis set (SASP) and per protocol analysis set (PPP) populations (Table 15). All subjects from the prospective phase met inclusion criterion 1, 17 (63.0%) subjects met inclusion sub-criterion 2.1, and 26 (96.3%) subjects met sub-criterion 2.2 (Annex 1.2: Table 14.1.7). No subject met the exclusion criterion (Annex 1.2: Table 14.1.8).

**Table 15 Prospective phase study population**

| | | ***Prospective Phase*** |
|---|---|---|
| FASP | No | 1 (3.70%) |
| | Yes | 26 (96.30%) |
| SASP | No | 1 (3.70%) |
| | Yes | 26 (96.30%) |
| PPP | No | 1 (3.70%) |
| | Yes | 26 (96.30%) |

Full Analysis Set for Prospective (FASP), Safety analysis set for Prospective data (SASP), Per Protocol analysis sets for Prospective data (PPP)

*Source: Table 14.1.6*

The mean (SD) time treated with BeneFIX was 1.5 (0.6) years (Table 16). Overall, 12 (46.2%) subjects completed the prospective follow-up phase and 14 (53.8%) discontinued the study. The majority of discontinued subjects (n=10; 76.9% of discontinued subjects) withdrew from the study as they had reached the administrative cut off limit (31$^{st}$ July 2016), one (7.7%) subject was withdrawn due to insufficient clinical response, one (7.7%) withdrew due to suspended treatment due to inhibitor development, and one (7.7%) withdrew due to starting another treatment (NovoSeven). The remaining discontinued subject (3.7%) stopped the study due to reaching six years of age.

**Table 16 Prospective phase subject disposition**

| | | ***Prospective Phase*** |
|---|---|---|
| Country | Total | 27 (100.00%) |
| | IT | 15 (55.56%) |
| | SP | 5 (18.52%) |
| | SW | 2 (7.41%) |
| | UK | 5 (18.52%) |
| Screening Failure | Total | 27 (100.00%) |
| | No | 26 (96.30%) |
| | Yes | 1 (3.70%) |
| Status subject | Total | 26 (100.00%) |
| | Completed 24 months of prospective observation | 12 (46.15%) |
| | Reached the age of 6 years | 1 (3.85%) |

| | | ***Prospective Phase*** |
|---|---|---|
| | Early termination | 13 (50.00%) |
| Early termination | Total | 13 (100.00%) |
| | Administrative cut off on 31st July 2016 | 10 (76.92%) |
| | Insufficient Clinical Response | 1 (7.69%) |
| | Other* | 2 (15.38%) |
| Time in prospective phase (years) | n | 26 |
| | N. missing | 0 |
| | Mean (95%CI) | 1.54 (1.30 , 1.77) |
| | Standard deviation (SD) | 0.59 |
| | Median | 1.91 |
| | (Q1, Q3) | (1.06, 2.00) |
| | (Min, Max) | (0.37, 2.15) |

(*) 1 subject suspends for inhibitor development, 1 subject starts NovoSeven
SF: Subject 1042-001 older than 5 years at the time of the inclusion into the Prospective Phase

*Source: Table 14.1.5*

## 10.2.2 Descriptive data

In the prospective phase of the study, the mean (SD) age of the FASP population was 2.5 (1.1) years old; 23 (88.5%) subjects were Caucasian (Table 17). All were male and 10 (38.5%) were attending school/kindergarten at study inclusion.

**Table 17 Full analysis set for prospective (FASP) phase: demographic data**

| | | ***FASP (N=26)*** |
|---|---|---|
| Ethnicity | | |
| | Caucasian | 23 (88.46%) |
| | Unknown | 1 (3.85%) |
| | Other | 2 (7.69%) |
| Age | n | 26 |
| | N. missing | 0 |
| | Mean (95%CI) | 2.48 (2.05 , 2.91) |
| | Standard deviation (SD) | 1.07 |
| | Median | 2.63 |
| | (Q1, Q3) | (1.64, 3.16) |
| | (Min, Max) | (0.27, 4.72) |
| Gender | | |
| | Male | 26 (100.00%) |
| School or kindergarten | | |
| | No | 11 (42.31%) |
| | Yes | 10 (38.46%) |
| | NA | 5 (19.23%) |

*Source: Table 14.1.15*

The mean (SD) age of diagnosis was 0.4 (0.4) years old and 16 (61.5%) subjects had a family history of haemophilia B. The mean age of first bleed related to haemophilia B was 0.6 (0.4) years old, and the age at which the subject was first exposed to BeneFIX was 0.9 (0.7) years old. The proportion of subjects with moderate to severe haemophilia B was

high with 20 (76.9%) reporting severe and five (19.2%) reporting moderate disease severity. Almost half of the subjects (n=12; 46.2%) were reported to have experienced at least one haemarthrosis, with the mean age (SD) being 1.3 (0.7) years old. Joints were not targeted for any subject, and 22 (84.6%) subjects were reported to have a factor IX gene mutation – the most common were missense mutations which were reported for eight (30.8%) subjects (Table 18).

**Table 18 FASP disease parameters**

| | | | ***FASP (N=26)*** |
|---|---|---|---|
| Age of diagnosis (years) | n | | 26 |
| | N. missing | | 0 |
| | Mean (95%CI) | | 0.38 (0.23 , 0.54) |
| | Standard deviation (SD) | | 0.38 |
| | Median | | 0.32 |
| | (Q1, Q3) | | (0.00, 0.72) |
| | (Min, Max) | | (0.00, 1.18) |
| Age of first bleed (years) | n | | 25 |
| | N. missing | | 1 |
| | Mean (95%CI) | | 0.63 (0.45 , 0.82) |
| | Standard deviation (SD) | | 0.44 |
| | Median | | 0.56 |
| | (Q1, Q3) | | (0.34, 1.00) |
| | (Min, Max) | | (0.00, 1.62) |
| Age at first exposure to BeneFIX (years) | n | | 26 |
| | N. missing | | 0 |
| | Mean (95%CI) | | 0.92 (0.63 , 1.22) |
| | Standard deviation (SD) | | 0.73 |
| | Median | | 0.83 |
| | (Q1, Q3) | | (0.50, 1.21) |
| | (Min, Max) | | (0.02, 3.74) |
| Family history | | | 26 (100.00%) |
| | Yes | | 16 (61.54%) |
| | No | | 10 (38.46%) |
| Severity of Haemophilia B | | | 26 (100.00%) |
| | Severe [FIX: C<1 IU/dL (%)] | | 20 (76.92%) |
| | Moderate [FIX: C 1 to 5 IU/dL (%)] | | 5 (19.23%) |
| | Mild [FIX: C >5 to 40 IU/dL (%)] | | 1 (3.85%) |
| Subjects with joint bleed | | | 26 (100.00%) |
| | No | | 14 (53.85%) |
| | Yes | | 12 (46.15%) |
| | Age at first joint bleed | n | 12 |
| | | N. missing | 0 |
| | | Mean (95%CI) | 1.33 (0.91 , 1.75) |
| | | Standard deviation (SD) | 0.66 |
| | | Median | 1.13 |
| | | (Q1, Q3) | (0.82, 1.73) |
| | | (Min, Max) | (0.59, 2.75) |
| Target joints | | | 26 (100.00%) |
| | No | | 24 (92.31%) |
| | NA | | 2 (7.69%) |
| Mutation | | | 26 (100.00%) |
| | Yes | | 22 (84.62%) |

| | | | ***FASP (N=26)*** |
|---|---|---|---|
| No | | | 4 (15.38%) |

*Source: Table 14.1.16*

BeneFIX was used as the first treatment for 23 (88.5%) subjects; the most common alternatives, used to treat two subjects each, were tranexamic acid (7.7%) and plasma (7.7%) [Table 19]..

**Table 19 FASP haemophilia B prior therapy**

| | | | ***FASP (N=26)*** |
|---|---|---|---|
| Did the subject receive BeneFIX as first treatment for Haemophilia B? | | | 26 (100.00%) |
| | Yes | | 23 (88.46%) |
| | No | | 3 (11.54%) |
| Did the subject receive any other product than BeneFIX for treatment of Haemophilia B? | | | 26 (100.00%) |
| | No | | 20 (76.92%) |
| | Yes | | 6 (23.08%) |
| | Preferred Name (*) | | |
| | | PLASMA | 2 (33.33%) |
| | | TRANEXAMIC ACID | 2 (33.33%) |
| | | EPTACOG ALFA (ACTIVATED) | 1 (16.67%) |
| | | FACTOR II (PROTHROMBIN) | 1 (16.67%) |
| | | FIX | 1 (16.67%) |
| | | INTRAFER TF | 1 (16.67%) |
| | | RED BLOOD CELLS | 1 (16.67%) |

(*) Percentage calculated on the total of subjects who received other product
Previous therapy for Haemophilia B coded using WHO-DDE version 16.1.

*Source: Table 14.1.17*

Two (7.7%) subjects had a history of inhibitors against FIX products, both related to BeneFIX (Listing 16.2.25.2). One event was of mild severity and one event was of severe severity; both patients temporarily stopped treatment for treatment of the inhibitors, before re-initiating treatment on an ITI regimen. Two (7.7%) subjects had a history of allergic reactions due to FIX products [Table 20]. Both subjects with allergic reactions were considered not related to BeneFIX treatment (sleepiness and hives and generalised urticaria) and both subjects experiencing FIX inhibitors were considered to be related to BeneFIX treatment. No RBC agglutination was documented in any subject.

**Table 20 FASP disease history**

| | | | ***FASP (N=26)*** |
|---|---|---|---|
| History of inhibitors | | | 26 (100.00%) |
| | Yes | | 2 (7.69%) |
| | No | | 24 (92.31%) |
| History of allergic reactions | | | 26 (100.00%) |
| | Yes | | 2 (7.69%) |
| | No | | 24 (92.31%) |
| | Signs or symptoms that resulted in diagnosis of allergic reactions(*) | | 2 (100.00%) |
| | | Hives | 1 (50.00%) |
| | | Generalized Urticaria | 1 (50.00%) |
| | | Sleepiness | 1 (50.00%) |
| RBC agglutination | | | 26 (100.00%) |
| | No | | 26 (100.00%) |

2 subjects have a history of inhibitor against BeneFIX products
[#1023-3 - Titres: 1.6; Peak: 2.2; Method of assessment: Bethesda; Lower detection value: 0.4]
[#1053-3 - Titres: 2.7, 25, 3.9, 10.4; Method of assesment: NA, Lower detection value: NA].
(*) Not mutually exclusive categories

*Source: Table 14.1.18*

For the FASP population, the medical history was collected for all subjects, with 13 (50.0%) having a significant medical history (Annex 1.2: Table 14.1.19). No subject had prior hepatitis A, B, or C viral infections; similarly, there were no subjects with HIV. For immunizations, 13 (50.0%) subjects were immunised against hepatitis A and B. Furthermore, there were 29 diseases/conditions, with the most common (surgical and medical procedures) being recorded in seven (26.9%) subjects. Prior and concomitant medication use was highly variable; the most common prior and concomitant medications were tranexamic acid (n=10; 38.5%) and paracetamol (n=4; 15.4%) [Annex 1.2: Table 14.1.20].

## 10.2.3 Efficacy Results

### 10.2.3.1 Annualised bleeding rates (ABR)

The mean (95% CI) ABR was 2.9 (0.9, 5.0) times per year (Table 21). At the beginning of the observation period, 17 (65.4%) subjects were receiving a prophylaxis regimen, seven (26.9%) were receiving an on-demand regimen and two (7.7%) were receiving an ITI regimen (Annex 1.2: Table 14.3.3.7). In the prospective phase a total of 19 (73.1%) subjects were categorised as receiving a prophylaxis regimen and five (19.2%) were categorised as receiving an on-demand regimen. The mean (95% CI) ABR for the 19 subjects mainly receiving a prophylaxis regimen was 2.9 (0.3, 5.4). The mean (95% CI) ABR for the five subjects receiving an on-demand regimen was 3.3 (0.0, 6.8). In all 24 subjects, the mean (95% CI) ABR related to traumatic bleeds was 2.2 (0.3, 4.2). The mean (95% CI) ABR related to spontaneous bleeds was 0.5 (0.1, 1.0). For soft tissue/muscle bleeds and joint bleeds the mean (95% CI) ABR were 0.9 (0.2, 1.7) and 0.4 (0.1, 0.8), respectively. The mean (95% CI) ABR for CNS bleeds was 0.1 (0.0, 0.1).

**Table 21 FASP annualised bleeding rates**

| | | ***FASP (N=26)*** |
|---|---|---|
| ABR (all bleeds) | n | 24 |
| | N. missing | 0 |
| | Mean (95%CI) | 2.94 (0.91 , 4.97) |
| | Standard deviation (SD) | 4.81 |
| | Median | 1.27 |
| | (Q1, Q3) | (0.00, 4.40) |
| | (Min, Max) | (0.00, 22.43) |
| ABR (prophylaxis regimen) | n | 19 |
| | N. missing | 0 |
| | Mean (95%CI) | 2.85 (0.31 , 5.38) |
| | Standard deviation (SD) | 5.26 |
| | Median | 1.05 |
| | (Q1, Q3) | (0.00, 3.00) |
| | (Min, Max) | (0.00, 22.43) |
| ABR (on-demand regimen) | n | 5 |
| | N. missing | 0 |
| | Mean (95%CI) | 3.29 (-0.24 , 6.82) |
| | Standard deviation (SD) | 2.84 |
| | Median | 4.54 |
| | (Q1, Q3) | (0.50, 5.24) |
| | (Min, Max) | (0.00, 6.18) |
| ABR (spontaneous bleeds) | n | 24 |
| | N. missing | 0 |
| | Mean (95%CI) | 0.54 (0.08 , 1.00) |
| | Standard deviation (SD) | 1.09 |
| | Median | 0.00 |
| | (Q1, Q3) | (0.00, 0.76) |
| | (Min, Max) | (0.00, 4.43) |
| ABR (traumatic bleeds) | n | 24 |
| | N. missing | 0 |
| | Mean (95%CI) | 2.21 (0.27 , 4.15) |
| | Standard deviation (SD) | 4.60 |
| | Median | 0.50 |
| | (Q1, Q3) | (0.00, 3.06) |
| | (Min, Max) | (0.00, 22.43) |
| ABR (joint bleeds) | n | 24 |
| | N. missing | 0 |
| | Mean (95%CI) | 0.43 (0.11 , 0.76) |
| | Standard deviation (SD) | 0.77 |
| | Median | 0.00 |
| | (Q1, Q3) | (0.00, 0.57) |
| | (Min, Max) | (0.00, 2.66) |
| ABR (soft tissue/muscle bleeds) | n | 24 |
| | N. missing | 0 |
| | Mean (95%CI) | 0.92 (0.19 , 1.65) |
| | Standard deviation (SD) | 1.74 |

| | | ***FASP (N=26)*** |
|---|---|---|
| | Median | 0.25 |
| | (Q1, Q3) | (0.00, 1.02) |
| | (Min, Max) | (0.00, 7.48) |
| ABR (CNS bleeds) | n | 24 |
| | N. missing | 0 |
| | Mean (95%CI) | 0.06 (-0.01 , 0.14) |
| | Standard deviation (SD) | 0.17 |
| | Median | 0.00 |
| | (Q1, Q3) | (0.00, 0.00) |
| | (Min, Max) | (0.00, 0.52) |
| ABR (Other bleeds) | n | 24 |
| | N. missing | 0 |
| | Mean (95%CI) | 1.52 (0.18 , 2.87) |
| | Standard deviation (SD) | 3.18 |
| | Median | 0.25 |
| | (Q1, Q3) | (0.00, 1.67) |
| | (Min, Max) | (0.00, 14.95) |

Subjects are classified into treatment groups (On-demand or Prophylaxis) based on the length of time on each regimen during the study phase:
On-demand, <50% of the treatment phase on prophylaxis; Prophylaxis, >= 50% of the treatment phase on prophylaxis. Three subjects have been treated with ITI after developing inhibitor (they have been classified in a separate category).
The subjects #1023-3 and #1053-3, who received an ITI regimen during the study period due to a history of BENEFIX inhibitors, are excluded from this table.

*Source: Table 14.2.1.5*

#### 10.2.3.2 Response to on-demand and prophylactic BeneFIX treatment

Sixteen (66.6%) subjects exhibited *Excellent* responses to BeneFIX treatment for on-demand bleeds in the prospective phase (Table 22). Eight (33.3%) subjects did not have a response collected. Stratification by treatment regimen showed that 12 (63.2%) and four (80.0%) subjects receiving prophylaxis or on-demand regimens (respectively) exhibited *Excellent* responses for on-demand treatment of bleeds with BeneFIX. The response was not collected in seven (36.8%) prophylaxis and one (10.0%) on-demand treatment regimen subjects.

**Table 22 FASP response to BeneFIX treatment**

| | | ***FASP (N=26)*** |
|---|---|---|
| Best Response (Overall) | | 24 (100.00%) |
| | Excellent (1) | 16 (66.67%) |
| | Good (2) | 0 (0.00%) |
| | Moderate (3) | 0 (0.00%) |
| | No response (4) | 0 (0.00%) |
| | Not applicable (5) | 0 (0.00%) |
| | Not collected | 8 (33.33%) |
| Best Response (Prophylaxis regimen) | | 19 (100.00%) |
| | Excellent (1) | 12 (63.16%) |
| | Good (2) | 0 (0.00%) |
| | Moderate (3) | 0 (0.00%) |
| | No response (4) | 0 (0.00%) |
| | Not applicable (5) | 0 (0.00%) |
| | Not collected | 7 (36.84%) |
| Best Response (On-demand regimen) | | 5 (100.00%) |
| | Excellent (1) | 4 (80.00%) |
| | Good (2) | 0 (0.00%) |
| | Moderate (3) | 0 (0.00%) |
| | No response (4) | 0 (0.00%) |
| | Not applicable (5) | 0 (0.00%) |
| | Not collected | 1 (20.00%) |

(1) Definite pain relief and/or improvement in signs of bleeding starting within 8 hours after an infusion with no additional infusion administered
(2) Definite pain relief and/or improvement in signs of bleeding starting within 8 hours after an infusion with at leat one additional infusion administered for complete resolution of bleeding episode. Or definite pain relief and/or improvement in signs of bleeding starting after 8 hours following the infusion with no additional infusion administered
(3) Probable or slight improvement starting after 8 hours following the infusion with at least one additional infusion administered for complete resolution of the bleeding episode
(4) No improvement at all between infusions or during the 24 hour interval following an infusion or condition worsens
Subjects are classified into treatment groups (On-demand or Prophylaxis) based on the length of time on each regimen during the study phase: On-demand, <50% of the treatment phase on prophylaxis; Prophylaxis, >= 50% of the treatment phase on prophylaxis. Three subjects have been treated with ITI after developing inhibitor (they have been classified in a separate category).
The subjects #1023-3 and #1053-3, who received an ITI regimen during the study period due to a history of BENEFIX inhibitors, are excluded from this table.

*Source: Table 14.2.1.6*

#### 10.2.3.3 Incidence of less-than-expected therapeutic effect (LETE)

No LETE was recorded in the prospective phase (Annex 1.2: Table 14.2.1.7)

### 10.2.3.4 FIX activity and recovery

In the prospective phase, FIX activity and recovery was recorded for 6 and 17 subjects, respectively (Table 23). Mean (95% CI) FIX activity was 2.9 (1.2, 4.6) IU/dL. Mean (95% CI) recovery of FIX:C was 0.5 (0.2, 0.8) IU/dL of FIX:C increase per IU/kg of BeneFIX administered.

**Table 23 FASP FIX activity and recovery**

| | | ***FASP (N=26)*** |
|---|---|---|
| Fix Activity (%) | n | 16 |
| | N. missing | 8 |
| | Mean (95%CI) | 6.06 (3.70 , 8.41) |
| | Standard deviation (SD) | 4.42 |
| | Median | 5.48 |
| | (Q1, Q3) | (2.34, 8.95) |
| | (Min, Max) | (0.80, 14.65) |
| Fix Activity (IU/dL) | n | 6 |
| | N. missing | 18 |
| | Mean (95%CI) | 2.89 (1.23 , 4.55) |
| | Standard deviation (SD) | 1.58 |
| | Median | 2.55 |
| | (Q1, Q3) | (2.20, 2.60) |
| | (Min, Max) | (1.45, 6.00) |
| Recovery | n | 7 |
| | N. missing | 17 |
| | Mean (95%CI) | 0.46 (0.15 , 0.76) |
| | Standard deviation (SD) | 0.33 |
| | Median | 0.56 |
| | (Q1, Q3) | (0.02, 0.66) |
| | (Min, Max) | (0.00, 0.90) |

The subjects #1023-3 and #1053-3 who received an ITI regimen during the study period due to a history of BENEFIX inhibitors are excluded from this table.

*Source: Table 14.2.2.3*

### 10.2.3.5 Daily Living impact

The daily living impact was measured in the prospective phase (Table24). During follow-up, the median (min - max) total number of days missed from work by parents or caregivers (n=10) was 0.0 (0.0 – 5.0) days. The median (min - max) total number of days when the subject was affected in daily activities by the disease (n=9) was 0.0 (0.0 - 5.0) days.

**Table 24 FASP daily living impact variables**

| | | ***FASP (N=26)*** |
|---|---|---|
| Total number of day missed from work by parents/caregivers | n | 10 |
| | N. missing | 14 |
| | Mean (95%CI) | 0.60 ( -0.53 , 1.73) |
| | Standard deviation (SD) | 1.58 |
| | Median | 0.00 |
| | (Q1, Q3) | (0.00, 0.00) |
| | (Min, Max) | (0.00, 5.00) |
| Total number of day when the subject was affected in daily activities due to his disease | n | 9 |
| | N. missing | 15 |
| | Mean (95%CI) | 0.76 ( -0.50 , 2.02) |
| | Standard deviation (SD) | 1.64 |
| | Median | 0.00 |
| | (Q1, Q3) | (0.00, 0.80) |
| | (Min, Max) | (0.00, 5.00) |

The subjects #1023-3 and #1053-3 who received an ITI regimen during the study period due to a history of BENEFIX inhibitors are excluded from this table.

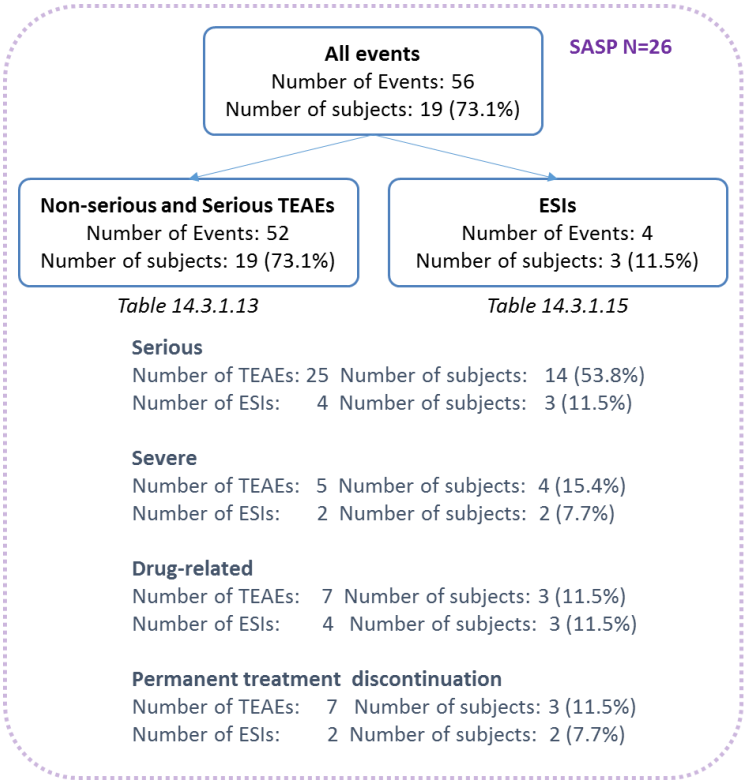
*Source: Table 14.2.2.2*

## 10.2.4 Safety results

### 10.2.4.1 Brief summary of safety results

The prospective phase safety analysis set consisted of 26 subjects. A total of 52 TEAEs were experienced by 19 (73.1%) subjects (Figure 5). Furthermore, 14 (53.8%) subjects had at least one treatment-emergent serious AE (TESAE), four (15.4%) subjects experienced at least one severe TEAE, and three (11.5%) subjects had a TEAE related to the BeneFIX treatment. Three (11.5%) subjects experienced TEAEs that led to permanent treatment discontinuation, two (7.7%) of these subjects also presented ESIs that led to treatment discontinuation. No deaths were reported. Three (11.5%) subjects experienced at least one ESI. All ESIs experienced in this prospective phase were considered to be serious and treatment-related. Two (7.7%) of the subjects discontinued the treatment due to an ESI (FIX inhibition and hypersensitivity) (Annex 1.2: Table 14.3.1.14). The individual lists of TEAEs and ESIs are provided in Annex 1.3: Listing 16.2.35 & 16.2.37.

**Figure 5 SASP summary of TEAEs**




### 10.2.4.2 Distribution of TEAEs by SOC and Preferred Term

A total of 52 TEAEs were experienced by 19 (73.1%) subjects (Table 25).

The most common SOCs for TEAEs were Injury, poisoning and procedural complications (16 TEAEs in nine [34.6%] subjects), Infections and infestations (10 TEAEs, six [23.1%] subjects); General disorders and administration site conditions (8 TEAEs, five [19.2%]

subjects), Gastrointestinal disorders (4 TEAEs, three [11.5%] subjects) and Blood and lymphatic system disorders (3 TEAEs, three [11.5%] subjects) (Table 25).

The most common Injury, poisoning and procedural complication TEAE, was Head injury, encountered by nine (34.6%) subjects and reported 13 times. The most frequently General disorders and administration site conditions TEAE was Pyrexia, reported five times by four (15.4%) subjects. The third more frequently reported PT was Anaemia (n=2 [7.7%]). All the other reported TEAEs by PT were encountered by one (3.9%) subject only (Table 25).

**Table 25 SASP TEAEs distributed by SOC and PT**

| | | ***SASP (N=26)*** | |
|---|---|---|---|
| | | **Number of Events** | **Number of Subjects** |
| **SOC** | **PT** | | |
| Number of Events / subjects with at least one treatment emergent adverse event | | 52 | 19 (73.08%) |
| Blood and lymphatic system disorders | | 3 | 3 (11.54%) |
| | Anaemia | 2 | 2 (7.69%) |
| | Neutrophilia | 1 | 1 (3.85%) |
| Cardiac disorders | | 1 | 1 (3.85%) |
| | Cyanosis | 1 | 1 (3.85%) |
| Gastrointestinal disorders | | 4 | 3 (11.54%) |
| | Mouth haemorrhage | 1 | 1 (3.85%) |
| | Tongue haemorrhage | 1 | 1 (3.85%) |
| | Vomiting | 2 | 1 (3.85%) |
| General disorders and administration site conditions | | 8 | 5 (19.23%) |
| | Fatigue | 1 | 1 (3.85%) |
| | Oedema peripheral | 1 | 1 (3.85%) |
| | Pyrexia | 5 | 4 (15.38%) |
| | Thermal burn | 1 | 1 (3.85%) |
| Infections and infestations | | 10 | 6 (23.08%) |
| | Candida infection | 1 | 1 (3.85%) |
| | Cellulitis | 1 | 1 (3.85%) |
| | Gastroenteritis | 2 | 1 (3.85%) |
| | Lower respiratory tract infection | 1 | 1 (3.85%) |
| | Respiratory infection | 1 | 1 (3.85%) |
| | Respiratory tract infection | 1 | 1 (3.85%) |
| | Rhinitis | 1 | 1 (3.85%) |
| | Viral infection | 1 | 1 (3.85%) |
| | Viral rash | 1 | 1 (3.85%) |

| | | ***SASP (N=26)*** | |
|---|---|---|---|
| | | **Number of Events** | **Number of Subjects** |
| **SOC** | **PT** | | |
| Injury, poisoning and procedural complications | | 16 | 9 (34.62%) |
| | Contusion | 1 | 1 (3.85%) |
| | Excoriation | 1 | 1 (3.85%) |
| | Face injury | 1 | 1 (3.85%) |
| | Head injury | 13 | 9 (34.62%) |
| Musculoskeletal and connective tissue disorders | | 1 | 1 (3.85%) |
| | Haemarthrosis | 1 | 1 (3.85%) |
| Nervous system disorders | | 1 | 1 (3.85%) |
| | Seizure | 1 | 1 (3.85%) |
| Product issues | | 1 | 1 (3.85%) |
| | Device issue | 1 | 1 (3.85%) |
| Renal and urinary disorders | | 2 | 2 (7.69%) |
| | Nephrotic syndrome | 1 | 1 (3.85%) |
| | Proteinuria | 1 | 1 (3.85%) |
| Respiratory, thoracic and mediastinal disorders | | 1 | 1 (3.85%) |
| | Cough | 1 | 1 (3.85%) |
| Skin and subcutaneous tissue disorders | | 2 | 1 (3.85%) |
| | Blister | 1 | 1 (3.85%) |
| | Erythema | 1 | 1 (3.85%) |
| Vascular disorders | | 2 | 2 (7.69%) |
| | Flushing | 1 | 1 (3.85%) |
| | Haematoma | 1 | 1 (3.85%) |

TEAEs = Number of treatment emengent adverse events in system organ class
Subjects who experienced multiple TEAEs within the same SOC and PT were counted only once, using the most sever e rating
TEAEs coded with MedDRA Dictionary version 19.0
This table includes only events that began during the study period

*Source: Table 14.3.1.13*

A total of five severe TEAEs were reported in four (15.4%) subjects in the prospective phase: Anaemia, Pyrexia, Oedema peripheral, Lower respiratory tract infection and Head injury (Annex 1.2: Table 14.3.1.16).

#### 10.2.4.3 Distribution of treatment related TEAEs by SOC and PT

A total of seven TEAEs related to BeneFIX were experienced by three (11.5%) subjects, all n=1 (3.9%): Cardiac disorders SOC (PT: Cyanosis), General disorders and administration site conditions SOC (PT: Fatigue), Renal and urinary disorders SOC (PT: Nephrotic syndrome), Respiratory, thoracic and mediastinal disorders SOC (PT: Cough), Skin and subcutaneous tissue disorders SOC (PTs: Blister, Erythema), and Vascular disorders SOC (PT: Flushing) [Annex 1.2: Table 14.3.1.18]. All the TEAEs related to BeneFIX were considered serious.

#### 10.2.4.4 Serious TEAESs

Fourteen (53.8%) subjects reported 25 TESAEs during the prospective phase of the study. The most common TESAEs distributed by SOC were Injury, poisoning and procedural complication (n=6, 23.1%) and Blood and lymphatic system disorder (n=2, 7.7%). The most common TESAE by PT was Head injury (n=6, 23.1%). The incidence of all the other reported TESAEs (Anaemia, Neutrophilia, Cyanosis, Mouth haemorrhage, Tongue haemorrhage, Fatigue, Oedema peripheral, Pyrexia, Lower respiratory tract infection, Contusion, Excoriation, Seizure, Device issue, Nephrotic syndrome, Cough, Blister, Erythema, Flushing and Hematoma) was of 3.9% (n=1) [Annex 1.2: Table 14.3.2.9].

Three (11.5%) subjects reported 7 treatment-related TESAEs during the prospective phase of the study, all n=1 (3.9%): Cyanosis, Fatigue, Nephrotic syndrome, Cough, Blister, Erythema and Flushing.

The dose was increased in 7 (26.9%) subjects due to a TESAE. TESAEs that led to a dose increase were Contusion, Excoriation, Head injury (in four different subjects), Oedema peripheral, Mouth haemorrhage and Tongue haemorrhage.

Additionally, three (11.5%) subjects permanently discontinued treatment due to a TSEAE. Subject #1019-3 discontinued BeneFIX due to Cough, Flushing and Fatigue of mild severity, and FIX inhibition (serious ESI described in Section 10.2.4.6). Subject #1023-3 discontinued due to a Nephrotic syndrome of moderate severity. The Nephrotic syndrome developed with massive proteinuria, low albumin, and high cholesterol most likely due to immune complexes by BeneFIX. Cholesterol was one of the main findings that support Nephrotic syndrome in children (due to protein loss). Nephrotic syndrome developed over weeks with around 20-30 injections of BeneFIX in that time period. The subject underwent laboratory tests and procedures which included albumin/creatinine ratio (range <3) at 418 g/mol (massive proteinuria) and urine protein at 3+ proteinuria (range low of 0); and albumin (range 35-47) at 22g/l. BeneFIX was permanently withdrawn on 19$^{th}$ January 2015. Subject #1053-3 discontinued treatment due to Erythema, Blister and Hypersensitivity (serious ESI described in Section 10.2.4.5).

## Table 26 SASP TESAEs

| SITE ID | PAT ID | Gender | Age | ESI | Term | SOC | PT | Onset | Resolved | Severity | Related | Action on Treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1018 | 4 | Male | 2.6 | No | SEVERE ANEMIA | Blood and lymphatic system disorders | Anemia | 16JUN2014 | 19JUN2014 | Severe | No | No action taken |
| 1019 | 3 | Male | 1.2 | No | COUGH | Respiratory, thoracic and mediastinal disorders | Cough | 28JUL2014 | 28JUL2014 | Mild | Yes | Permanently discontinued |
| | | | | No | CRANIAL TRAUMA | Injury, poisoning and procedural complications | Head injury | 07APR2015 | 08APR2015 | Mild | No | Permanently discontinued |
| | | | | No | FLUSHING ON FACE AND ARMS | Vascular disorders | Flushing | 28JUL2014 | 28JUL2014 | Mild | Yes | Permanently discontinued |
| | | | | Yes | INHIBITOR DEVELOPING | Blood and lymphatic system disorders | Factor IX inhibition | 30JUL2014 | UNK | Severe | Yes | Permanently discontinued |
| | | | | No | PROSTRATION | General disorders and administration site conditions | Fatigue | 28JUL2014 | 28JUL2014 | Mild | Yes | Permanently discontinued |
| 1020 | 1 | Male | 3.5 | No | CONTUSION | Injury, poisoning and procedural complications | Contusion | 08AUG2014 | 16AUG2014 | Mild | No | Dose Increased |
| | | | | No | EXCORIATION | Injury, poisoning and procedural complications | Excoriation | 08AUG2014 | 16AUG2014 | Mild | No | Dose Increased |
| | | | | No | HEAD TRAUMA | Injury, poisoning and procedural complications | Head injury | 08DEC2013 | 16DEC2013 | Moderate | No | Dose Increased |
| 1023 | 3 | Male | 3.1 | Yes | ALLERGIC REACTION | Immune system disorders | Hypersensitivity | 06APR2014 | 07APR2014 | Mild | Yes | No action taken |

| SITE ID | PAT ID | Gender | Age | ESI | Term | SOC | PT | Onset | Resolved | Severity | Related | Action on Treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No | NEPHROTIC SYNDROME-GLOMERULONEPHRITIS | Renal and urinary disorders | Nephrotic syndrome | 19JAN2015 | UNK | Moderate | Yes | Permanently discontinued |
| 1025 | 2 | Male | 0.5 | No | EDEMA IN LEFT FOREARM | General disorders and administration site conditions | Oedema peripheral | 13APR2016 | 19APR2016 | Severe | No | Dose Increased |
| 1026 | 1 | Male | 2.1 | No | HEAD TRAUMA | Injury, poisoning and procedural complications | Head injury | 28AUG2015 | 31AUG2015 | Moderate | No | Dose Increased |
| | 2 | Male | 2.6 | No | BLEEDING TRAUMA MOUTH | Gastrointestinal disorders | Mouth haemorrhage | 23MAR2016 | 30MAR2016 | Mild | No | Dose Increased |
| | | | | No | HAEMATOMA ON FOREHEAD | Vascular disorders | Haematoma | 01MAY2016 | 13MAY2016 | Mild | No | No action taken |
| 1030 | 2 | Male | 3.2 | No | SEIZURES | Nervous system disorders | Seizure | 22MAR2016 | 23MAR2016 | Moderate | No | No action taken |
| 1032 | 1 | Male | 1.6 | No | HEAD BUMP | Injury, poisoning and procedural complications | Head injury | 30JUN2016 | 01JUL2016 | Mild | No | No action taken |
| | 2 | Male | 3.2 | No | CHEST INFECTION | Infections and infestations | Lower respiratory tract infection | 20MAR2016 | 24MAR2016 | Severe | No | No action taken |
| 1043 | 1 | Male | 2.7 | No | TONGUE BLEEDING FOR BITE | Gastrointestinal disorders | Tongue haemorrhage | 12NOV2015 | 17NOV2015 | Mild | No | Dose Increased |
| | 2 | Male | 1.2 | No | CRANIAL TRAUMA | Injury, poisoning and procedural complications | Head injury | 22MAY2014 | 26MAY2014 | Mild | No | Dose Increased |
| | 4 | Male | 0.3 | No | CRANIC TRAUMA | Injury, poisoning and procedural complications | Head injury | 11DEC2014 | 12DEC2014 | Severe | No | Dose Increased |
| 1053 | 3 | Male | 2.4 | Yes | ALLERGIC REACTION | Immune system disorders | Hypersensitivity | 19NOV2013 | 19NOV2013 | Severe | Yes | No action taken |

| SITE ID | PAT ID | Gender | Age | ESI | Term | SOC | PT | Onset | Resolved | Severity | Related | Action on Treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Yes | ALLERGIC REACTION | Immune system disorders | Hypersensitivity | 24FEB2014 | 24FEB2014 | NA | Yes | Permanently discontinued |
| | | | | No | CYANOSIS | Cardiac disorders | Cyanosis | 19NOV2013 | 19NOV2013 | NA | Yes | No action taken |
| | | | | No | ERYTHEMA | Skin and subcutaneous tissue disorders | Erythema | 24FEB2014 | 24FEB2014 | NA | Yes | Permanently discontinued |
| | | | | No | FEVER | General disorders and administration site conditions | Pyrexia | 27NOV2013 | 29NOV2013 | NA | No | NA |
| | | | | No | NEUTHROPHILIC LEUKOCYTOSIS | Blood and lymphatic system disorders | Neutrophilia | 29NOV2013 | 29NOV2013 | NA | No | NA |
| | | | | No | PORTH OBSTRUCTION | Product issues | Device issue | 23NOV2013 | 25NOV2013 | NA | No | No action taken |
| | | | | No | VESICLES | Skin and subcutaneous tissue disorders | Blister | 24FEB2014 | 24FEB2014 | NA | Yes | Permanently discontinued |

This table includes only events that began during the study period

*Source: Table 14.3.2.4*

### 10.2.4.5 Events of special interest (ESI)

There were four ESIs experienced by three (11.5%) subjects – one case of Factor IX inhibition in one (3.9%) subject and three cases of Hypersensitivity reported in two (7.7%) subjects (Table 27). All the ESI reported were considered to be serious (SESIs) and are described in the next section (Table 28). The IDR (95% CI) for the individual ESIs in the prospective study was 0.108 (0.04, 0.25) (Annex 1.2: Table 14.3.1.22).

The severity of the Hypersensitivity ESIs was documented as severe, mild and unknown, respectively(Annex 1.2: Table 14.3.1.17). Factor IX inhibition was documented as of severe severity. All ESIs were related to BeneFIX (Annex 1.2: Table 14.3.1.19). At the end of follow-up, Hypersensitivity ESIs in two (7.7%) subjects were resolved and the Factor IX inhibition ESI was not resolved (Annex 1.2: Table 14.3.1.21). Two (7.7%) subjects were permanently discontinued due to an ESI (Factor IX inhibition and Hypersensitivity) (Annex 1.3: Listing 16.2.37).

**Table 27 SASP number of subjects with ESIs by SOC and PT**

| | | *SASP (N=26)* | |
|---|---|---|---|
| | | **Number of events** | **Number of Subjects** |
| **SOC** | **PT** | | |
| Number of ESIs / subjects with at least one event of special interest | | 4 | 3 ( 11.54%) |
| Blood and lymphatic system disorders | | 1 | 1 (3.85%) |
| | Factor IX inhibition | 1 | 1 (3.85%) |
| Immune system disorders | | 3 | 2 (7.69%) |
| | Hypersensitivity | 3 | 2 (7.69%) |

ESIs = Events of special interest
Subjects who experienced multiple ESIs within the same SOC and PT were counted only once, using the most severe rating
ESI coded with MedDRA Dictionary version 19.0
This table includes only events that began during the study period

*Source: Table 14.3.1.15*

### 10.2.4.6 Serious events of special interest

Subject #1019-3 experienced a Factor IX inhibition, subject #1023-3 experienced Hypersensitivity, and subject #1053-3 experienced two Hypersensitivity events. Details related to the Factor IX inhibition event is detailed below.

**AER Identifier(s): 2014219650**
**Subject: #1019-3**
**Serious events of special interest (system organ class [preferred term]): Blood and lymphatic system disorders (Factor IX inhibition)**

Subject #1019-3 was a 1.2 year old Caucasian male with severe haemophilia (FIX: C <1% IU/dl), a family history of haemophilia, and no significant history, inhibitors, or allergic reactions prior to BeneFIX treatment. The subject had factor IX gene mutation (Exons G and H of F9 deletion) and the subject's medical history included an oral frenectomy. The

subject was included in the prospective phase of the study on the 11th December 2013 and started to receive BeneFIX from 02nd December 2013 (on-demand regimen).

On the 30th July 2014, at 1 year of age, the subject developed factor IX inhibitors after receiving 12 infusions (12 exposure days) of BeneFIX (on-demand BeneFIX regimen in the event of bleeding events). The first positive titre was on 30th July 2014 (16.3 BU, and 9.7 BU on 18th August 2014). The event term was provided by investigator as inhibitor developing with onset date 30th July 2014 which was medically significant. On 28th July 2014, the subject presented flushing on the face and arms, cough and prostration during BeneFIX infusion. Symptoms were resolved after a few minutes and the subject remained under medical observation for some hours but no further symptoms developed. In response to these ESIs, BeneFIX was permanently withdrawn on 28th July 2014. The factor IX inhibition was not resolved at the end of the study period. The subject prematurely discontinued the study on the 08th April 2015 due to Cranial trauma (07th April 2015) that resulted in treatment with another medication.

## Table 28 SASP details of ESIs

| SITEID | SUBJID | Term<br>TEAE<br>Serious<br>SOC and PT | Onset | Date resolved | Severity | Related | Cause | Concomitant Treatment | Other Illness | Other | Action on Treatment | Subject | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1019 | 3 | INHIBITOR DEVELOPING<br>Yes<br>Yes<br>Blood and lymphatic system disorders<br>Factor IX inhibition | 30/07/2014 | UNK | Severe | Yes | | | | | Permanently discontinued | Action on subject - No action | Not Resolved |
| 1023 | 3 | ALLERGIC REACTION<br>Yes<br>Yes<br>Immune system disorders<br>Hypersensitivity | 06/04/2014 | 07/04/2014 | Mild | Yes | | | | | No action taken | Action on subject - Treatment given | Resolved |
| 1053 | 3 | ALLERGIC REACTION<br>Yes<br>Yes<br>Immune system disorders<br>Hypersensitivity | 19/11/2013 | 19/11/2013 | Severe | Yes | | | | | No action taken | Action on subject - No action | Resolved |
| 1053 | 3 | ALLERGIC REACTION<br>Yes<br>Yes<br>Immune system disorders<br>Hypersensitivity | 24/02/2014 | 24/02/2014 | NA | Yes | | | | | Permanently discontinued | Action on subject - Withdrawn from study | Resolved |

*Source: Listing 16.2.37*

#### 10.2.4.1 Lack of effect

In the prospective phase, there were no cases of a lack of effect recorded for any subject (Annex 1.2: Table 14.2.1.8).

# 11 Discussion

## 11.1 Key results

### 11.1.1 Study population and demographic data

This is a two-phase, non-interventional and multicentre registry study including a retrospective and prospective data collection phase. From the 38 paediatric subjects who were eligible for inclusion in the retrospective phase, 37 subjects were included in the retrospective phase analysis and 17 subjects continued to the prospective phase of the study. Table 29 provides a comparison of both the retrospective and prospective subject populations.

**Table 29 Key demographic results for both phases**

| Demographic Variable | FAS-Retrospective (N=37) | FAS-Prospective (N=26) |
|---|---|---|
| Age, mean (SD) | 5.0 (2.1) | 2.5 (1.1) |
| Gender, male, n (%) | 37 (100.0) | 26 (100.0) |
| Ethnicity, Caucasian, n (%) | 34 (91.9) | 23 (88.5) |
| Age of diagnosis (years), mean (SD) | 0.5 (0.7) | 0.4 (0.4) |
| Age of first bleed (years), mean (SD) | 0.8 (0.8) | 0.6 (0.4) |
| Age at first BeneFIX exposure (years), mean (SD) | 1.1 (0.8) | 0.9 (0.7) |
| BeneFIX as the first treatment for Haemophilia B, n (%) | 32 (86.5) | 23 (88.5) |
| Family history of Haemophilia B, n (%) | 25 (67.6) | 16 (61.5) |
| Severe severity of Haemophilia B, n (%) | 25 (67.6) | 20 (76.9) |

*Source: Table 14.1.9, 14.1.10, 14.1.15, 14.1.16*

All of the subjects that entered the study were male, mostly Caucasian (91.9% and 88.5% in the retrospective and prospective phases, respectively), and diagnosed with Haemophilia B at around 6 months of age (0.5 vs. 0.4 years). The age of first bleed was several months after diagnosis (0.8 vs. 0.6 years), and the first exposure to BeneFIX was around one year of age (1.1 vs. 0.9 years), and BeneFIX was used as the first treatment for most (86.5% vs. 88.5%) of the subjects. Most (67.6% vs. 61.5%) subjects had a family history of haemophilia B and most (67.6% vs. 76.9%) suffered from severe severity Haemophilia B.

### 11.1.2 Efficacy results

The primary objective of the EUREKIX study was to collect safety data related to the use of BeneFIX in children aged below 6 years of age and who were treated in a routine clinical practice setting. Secondary objectives included: the ABR, the response to on-demand and prophylactic BeneFIX treatment, LETE, lack of effect and daily living impact.

The mean ABR was 3.4 and 2.9 times in the retrospective and prospective study phases, respectively (Table 30). The mean ABR for subjects receiving BeneFIX on a prophylaxis regimen was 3.7 and 2.9 times, respectively. The ABR for on-demand regimen subjects was 3.0 and 3.3 times, respectively. The mean ABR for spontaneous bleeds and traumatic bleeds was 0.5 and 2.2 (respectively) for both the retrospective and prospective phases. The investigators' assessment of best response to BeneFIX treatment was almost all *Excellent* or *Good*. FIX activity was a respective 26.0 and 2.9 IU/dL; and FIX recovery was between 1.4 and 0.5 IU/dL.

One LETE occurred in the retrospective phase in the prophylaxis setting. There were three cases of a lack of effect documented in two subjects in the retrospective phase, all of mild severity. Two events were considered to be related to BeneFIX and in all three cases, the dose of BeneFIX was increased and no further action was required. No cases were documented in the prospective phase.

**Table 30 Key efficacy results for both phases**

| Efficacy Variable | FAS-Retrospective (N=35) | FAS-Prospective (N=26) |
|---|---|---|
| ABR, mean (95% CI) | | |
| All bleeds | 3.4 (2.5, 4.4) | 2.9 (0.9, 5.0) |
| Prophylaxis regimen | 3.7 (2.7, 4.7) | 2.9 (0.3, 5.4) |
| On-demand regimen | 3.0 (0.8, 5.1) | 3.3 (0.0, 6.8) |
| Spontaneous bleeds | 0.5 (0.3, 0.7) | 0.5 (0.1, 1.0) |
| Traumatic bleeds | 2.2 (1.5, 2.9) | 2.2 (0.3, 4.2) |
| Joint bleeds | 0.6 (0.4, 0.8) | 0.4 (0.1, 0.8) |
| Soft tissue/muscle bleeds | 1.1 (0.6, 1.6) | 0.9 (0.2, 1.7) |
| CNS bleeds | 0.1 ( 0.0, 0.1) | 0.1 (0.0, 0.1) |
| Best response as *Excellent* or *Good*, n (%) | | |
| Overall | 34 (97.1) | 16 (100.0)* |
| Prophylaxis regimen | 24 (100.0) | 12 (100.0)* |
| On-demand regimen | 10 (90.9) | 4 (100.0)* |
| Less-than-expected therapeutic effect, n (%) | 1 (2.7) | 0 (0.0) |
| Lack of effect, n (%) | 2 (5.4) | 0 (0.0) |
| FIX activity and recovery, IU/dL, mean (95% CI) | | |
| Activity | 26.0 (5.2, 46.7) | 2.9 (1.2, 4.6) |
| Recovery | 1.4 (0.0, 3.0) | 0.5 (0.2, 0.8) |

* Percentage of subjects calculated from collected responses

*Source: Table 14.2.1.1 – 14.2.1.8, 14.2.2.1, 14.2.2.3*

The daily living impact was measured in the prospective phase. During follow-up, the median (min - max) total number of days missed from work by parents or caregivers (n=10) was 0.0 (0.0 – 5.0) days. The median (min - max) total number of days when the subject was affected in daily activities by the disease was 0.0 (0.0 - 5.0) days.

### 11.1.3 Safety results

In the retrospective phase two ADRs related to BeneFIX (Ecchymosis and Skin haemorrhage) were recorded for one (2.7%) subject; both of mild severity and resolved by the end of this study phase (Table 31). There were four ESIs (Hypersensitivity) experienced by one (2.7%) subject, all of which were considered to be SESIs and related to BeneFIX; no ESIs led to permanent treatment discontinuation. No deaths were reported in this retrospective phase.
During the prospective phase, the most common TEAEs by SOC were: Injury, oisoning and procedural complications (n=9 [34.6%]); Infection and infestations (n=6 [23.1%]); General disorders and administration site conditions (n=5 [19.2%]); Gastrointestinal disorders (n=3 [11.5%],) and Blood and lymphatic system disorders (n=3 [11.5%]. The most common (> 10%) TEAE by PT were Head injury (n=9 [34.6%]) and Pyrexia (n=4 [15.4%]). Three (11.5%) subjects had at least one TEAE related to BeneFIX, (all n=1 [3.9%]): Cyanosis, Fatigue, Nephrotic syndrome, Cough, Blister, Erythema and Flushing.

Fourteen (53.8%) subjects experienced at least one TESAE. The most common TESAE by PT was Head injury (n=6; 23.1%). The incidence of all the other reported TESAE (Anaemia, Neutrophilia, Cyanosis, Mouth haemorrhage, Tongue haemorrhage, Fatigue, Oedema peripheral, Pyrexia, Lower respiratory tract infection, Contusion, Excoriation, Seizure, Device issue, Nephrotic syndrome, Cough, Blister, Erythema, Flushing and Hematoma) was of 3.9% (n=1). Furthermore, three (11.5%) subjects reported at least one treatment-related TESAE, all n=1 (3.9%): Cyanosis, Fatigue, Nephrotic syndrome, Cough, Blister, Erythema and Flushing. The dose was increased in seven (26.9%) subjects due to a TESAE. Additionally, three (11.5%) subjects permanently discontinued treatment due to a TESAE; one (3.8%) subject due to Cough, Flushing and Fatigue of mild severity and FIX inhibition of severe severity; one (3.8%) due to a nephrotic syndrome of moderate severity; and one (3.8%) due to Hypersensitivity, Erythema and Blisters of unknown severity.

In terms of ESIs, three (11.5%) subjects experienced at least one ESI, all were considered to be SESIs. There was one case of FIX inhibition of severe severity in one (3.9%) subject, and three cases of Hypersensitivity reported for two (7.7%) subjects. All ESIs were considered treatment related; two (7.7%) subjects discontinued treatment due to an ESI (FIX inhibition and Hypersensitivity). No deaths were reported in this prospective phase.

**Table 31 Key safety results for both phases**

| Safety Variable | SAS-Retrospective (N=37) | SAS-Prospective (N=26) |
|---|---|---|
| Subjects with ADRs/TEAEs, n (%) | 1 (2.7) | 19 (73.1) |
| Number of ADRs/TEAEs | 2 | 52 |
| Treatment-related | 2 | 7 |
| Subjects with SADRs/TESAEs, n (%) | 0 (0.0) | 14 (53.8) |
| Number of SADRs/TESAEs | 0 | 25 |
| Number of SADRs/TESAEs of severe severity | 0 | 5 |
| Subjects with ESIs (all serious), n (%) | 1 (2.7) | 3 (11.5) |
| Number of ESIs | 4 | 4 |
| Factor IX inhibition | 0 | 1 |
| Hypersensitivity | 4 | 3 |
| Subjects with ESIs leading to permanent treatment discontinuation, n (%) | 0 (0.0) | 2 (7.7) |

*Source: Table 14.3.1.1 – 14.3.1.22*

Overall, in this study there was one subject in the prospective phase (1/46 unique subject; 2.2%) with a Factor IX inhibition SESI. The subject presented with severe Haemophilia B, was considered to be a previously untreated patient (PUP) with no prior exposure to FIX replacement prior to BeneFIX. Furthermore the subject had a factor IX gene mutation (deletion), no family history of haemophilia B and no history of inhibitors.

Seven Hypersensitivity ESIs was experienced by three subjects (3/46 unique subjects; 6.5%); four were of mild severity, two were of severe severity, and one was unknown.

## 11.2 Limitations

The study was designed taking into account the long duration of this disease and its low incidence. However, as for any study, there are limitations inherent to the study design. In the retrospective phase of the study, safety and efficacy were measured based on medical history, thus giving limited control over the data collection as it was gathered retrospectively.

Furthermore, the voluntary participation of investigators constituted a selection bias observed for this type of study. Another potential selection bias classically associated with non-interventional studies was subject selection. Voluntary or involuntary selection of subjects in a study by investigators is inevitable, but this bias was limited by systematic attempts of the investigators to enrol subjects in the study. The pragmatic nature of this study, which involved routine clinical care management practices, complicated the collection of follow-up data and may have increased the number of subjects lost to follow-up. Differential losses to follow up can also lead to bias in retrospective cohort studies especially as the retrospective to prospective comparison were not performed in the same subjects throughout the study,

rather both groups were analysed separately. Finally, the conclusions drawn from this study are limited by the low number of subjects included in the study due to the low incidence of haemophilia B.

## 11.3 Interpretation

The primary objective of this two phase non-interventional, multicentre registry study was to assess the safety of BeneFIX in subjects below six years of age. In the retrospective phase ADRs and ESIs related to BeneFIX were collected. Two ADRs and four SESIs, all of mild severity, were recorded. By contrast, in the prospective phase, all TEAEs and ESIs were recorded, regardless of relation to BeneFIX. A total of 52 TEAEs, 25 TESAEs and four ESIs were recorded in this phase. Differences in data collection methodologies and time of follow-up make direct comparisons between phases challenging. Overall, the adverse events assessed as related to treatment with BeneFIX and reported in each of the phases in this study were consistent with the profile observed in children and adults exposed to the product in other studies conducted by the marketing authorisation holder in addition to the information accrued with BeneFIX through market exposure. No new safety concerns were detected in this study with a safety profile commensurate with that communicated through the product EU SmPC.[1] The efficacy secondary objective of this study was achieved by assessing ABRs, responses to treatment regimens, the incidence of LETEs and the lack of treatment effect. Overall ABR rates in both phases of this study were similar to the results from Mohanan *et al.* 2010, a prior study of BeneFIX in 25 children <6 years of age with severe haemophilia in a routine clinical practice setting.[5] Likewise, compared to a recent retrospective analysis of haemophilia B subjects (both adults and children from six EU countries) of varying disease severity (ranging from 14.3% in Belgium to 74.0% in Germany) the ABRs for both the retrospective and prospective phases of this study were within the ABR range observed for moderate-to-severe haemophilia B sufferers (range of means: 1.7 to 8.5).[7] Unlike previous BeneFIX studies, differences in the on-demand ABR between prophylaxis vs. on-demand regimens were observed to be substantially smaller.[8,9] As subjects included in this study were able to transition between regimens, it is likely that any true underlying differences in ABRs between treatment regimens were obscured.

The response to treatment of subjects in the present study was in alignment with prior BeneFIX studies in adults and children; on-demand infusion responses in subjects receiving either a prophylaxis or on-demand treatment regimen were considered to be Excellent or Good in both phases of this study.[5,8,10] The paucity of prior data collected for LETEs or lack of effect endpoints, in children or otherwise, prevents any direct comparison or any substantive conclusions. In this study one subject experienced a LETE and two subjects exhibited a lack of effect; all three were in the retrospective phase. This was higher than the incidence reported for moderately-severe to severe adult subjects where no LETEs or lack of effect were observed.[8] Possible reasons for the higher incidence of these outcomes in this study include the inclusion of predominantly untreated paediatric population with greater variability in baseline characteristics.

## 11.4 Generalisability

The results from this two-phase study included paediatric cohorts of subjects of varying haemophilia B severity from four European countries. The wide eligibility criteria, with only those receiving treatment other than BeneFIX being excluded from participation, affords a high degree of external validity in predominantly Caucasian nations with equivalent standards of life. Nevertheless, the absence of ethnic diversity and healthcare system models included

in this study are important limitations for the generalisability of these results to the global paediatric population of haemophilia B sufferers.

# 12 Other information

Not applicable

# 13 Conclusion

This post-authorisation safety study on the use of BeneFIX for the treatment of haemophilia B in routine clinical practice provides safety and efficacy data from subjects receiving treatment in four European countries – Italy, Spain, Sweden and the UK. Overall, the adverse events reported as related to BeneFIX in each of the study phases was consistent with the profile observed to-date in adults and children treated with BeneFIX and did not suggest any new safety concern. In relation to efficacy, overall results are mostly supported by data from prior studies; however, the absence of pooled data assessments limits the conclusions that can be drawn from this study in the context of available evidence. Nevertheless, ABRs and responses to treatment regimens were similar to previous BeneFIX studies. Given the routine clinical practice setting of this study with wide eligibility criteria, a high degree of external validity can be assumed for predominantly Caucasian individuals with equivalent standards of life. The absence of ethnic diversity and healthcare system models included in this study are important limitations for the generalisability of these results to the global paediatric population of haemophilia B sufferers.

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# 15 Appendices

## Annex 1. List of stand-alone documents

Documents listed in Annex 1. can be maintained separately from the study final study report. They should be clearly identifiable and provided on request. Write “None” if there is no document or list documents in a table as indicated below.

| Number | Document reference number | Date | Title |
|---|---|---|---|
| 1 | Annex 1.1 | 11 APR 2017 | Annex 1.1 Signature page |
| 2 | Annex 1.2 | 26 JAN 2018 | Annex 1.2 Tables & Figures |
| 3 | Annex 1.3 | 26 JAN 2018 | Annex 1.3 Listings |
| 4 | Annex 1.4 | 21 MAR 2016 | Annex 1.4 Study Protocol |
| 5 | Annex 1.5 | 22 JAN 2016 | Annex 1.5 Statistical Analysis Plan |

## Annex 2 Additional information

Not applicable.