Recombinant factor XIII: a safe, and novel treatment for congenital factor XIII deficiency

Aida Inbal¹, Johannes Oldenburg², Manuel Carcao³, Anders Rosholm⁴, Ramin Tehranchi⁵, Diane Nugent⁶

¹Thrombosis and Hemostasis Unit Rabin Medical Center, Petah Tikva and Sackler Faculty of Medicine, Tel Aviv University, Israel; ²Institute of Experimental Haematology and Transfusion Medicine, University Clinic, Bonn, Germany; ³Haematology/Oncology, Hospital for Sick Children, Toronto, Canada; ⁴Biostatistics, Novo Nordisk A/S, Copenhagen, Denmark; ⁵Medical and Science, Haemostasis R&D Portfolio, Novo Nordisk A/S, Copenhagen, Denmark; ⁶Hematology, CHOC Children’s Hospital/UC Irvine Medical School, Orange, CA, USA

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Corresponding author:

Diane Nugent, Hematology, CHOC Children’s Hospital/ UC Irvine Medical School, Orange, CA, USA.

Email: dnugent@choc.org

Telephone: +1 (714) 532-8459

Fax: +1 (714) 532-8698
Abstract

Congenital factor XIII deficiency is a rare autosomal recessive disorder with most patients having an A subunit (FXIII-A) deficiency. Patients experience life-threatening bleeds, impaired wound healing, and spontaneous abortions. In many countries, only plasma or cryoprecipitate treatments are available, but these carry a risk for infection with blood-borne pathogens and allergic reactions. A multinational, open-label, single-arm, phase 3 prophylaxis trial evaluated the efficacy and safety of a novel recombinant FXIII (rFXIII) in congenital FXIII-A subunit deficiency. Forty-one patients aged ≥6 years (mean: 26.4 [range: 7-60] years) with congenital FXIII-A subunit deficiency were enrolled. Throughout the rFXIII prophylaxis, only 5 bleeding episodes (all trauma-induced) in 4 patients were treated with FXIII-containing products. Crude mean bleeding rate was significantly lower than the historic bleeding rate (0.138 bleeds/patient/year versus 2.91, respectively) for on-demand treatment. Transient, non-neutralizing, low-titer anti-rFXIII antibodies developed in 4 patients, none of whom experienced allergic reactions, any bleeds requiring treatment, or changes in FXIII pharmacokinetics during the trial or follow-up. These non-neutralizing antibodies declined below detection limits in all 4 patients, despite further exposure to rFXIII/other FXIII-containing products. In conclusion, rFXIII is safe and effective in preventing bleeding episodes in patients with congenital FXIII-A subunit deficiency.

(http://www.clinicaltrials.gov: NCT00713648.)
Introduction

Factor XIII (FXIII) is a protransglutaminase that, following activation by thrombin and the presence of calcium, becomes transglutaminase; this cross-links γ-glutamyl-ε-lysine residues of fibrinogen chains leading to increased stability of the fibrin clot. Plasma FXIII is a heterotetramer composed of 2 catalytic A subunits and 2 carrier B subunits linked by noncovalent bonds. The average plasma concentration of A2B2 heterotetramer is approximately 22 µg/mL and its half-life is 9-14 days.1

Congenital FXIII deficiency is a severe bleeding disorder transmitted in an autosomal recessive manner. Typical bleeding manifestations include umbilical stump bleeding during the first few days of life, postoperative bleeding, and intracranial hemorrhage, which is observed more frequently in FXIII deficiency than in other inherited bleeding disorders. In addition, FXIII deficiency is associated with recurrent pregnancy losses and delayed wound healing.2 Most congenital FXIII deficiency is caused by FXIII-A subunit deficiency; this occurs at a frequency of approximately 1 in 2 million,3 with 50% of the molecular defects responsible for A subunit deficiency being missense mutations.2 Congenital deficiency of the FXIII-B subunit is a rare cause of clinically significant FXIII deficiency.4

The severity of bleeding symptoms in congenital FXIII deficiency is the main reason for regular replacement therapy. Prophylaxis is highly efficient and successful due to the long half-life of FXIII. To date, only plasma-derived sources of FXIII have been available,5,6 including fresh frozen plasma, cryoprecipitate, and a plasma-derived, virally inactivated FXIII concentrate.

A new recombinant FXIII (rFXIII), originally developed by ZymoGenetics Inc, and later transferred to Novo Nordisk (Novo Nordisk A/S, Copenhagen, Denmark), has been manufactured in Saccharomyces cerevisiae (yeast) and contains no human/mammalian products.7 The rFXIII associates in plasma with the endogenous FXIII-B subunit to form the stable FXIII heterotetramer. In a phase 1 clinical trial, rFXIII had
a half-life similar to that of native FXIII. This new product was found to have a good safety profile and is appropriate for development for monthly prophylactic administration in patients with FXIII-A subunit deficiency. Therefore, a multinational, open-label, single-arm, multiple-dosing, phase 3 prophylaxis trial was undertaken to evaluate the efficacy and safety of rFXIII for the prevention of bleeding in congenital FXIII-A subunit deficiency.

Material and methods

Study design

The study was approved by Ethical Committees at each participating hospital. Informed consent was obtained from each patient in accordance with the Declaration of Helsinki.

In total, 41 patients were enrolled from 23 centers in 11 countries (Austria, Canada, Finland, France, Germany, Israel, Italy, Spain, Switzerland, UK, and USA). Following a screening visit, eligible patients entered a 4-week run-in period followed by a 52-week treatment period (visits 2-15) of monthly (28±2 days) doses of 35 IU/kg rFXIII intravenously. Patients receiving prophylaxis with a FXIII-containing product before the trial received their last prophylactic dose just prior to screening. Only patients with a confirmed FXIII-A subunit deficiency were given rFXIII at visit 2 (week 4). At each visit, the dose was adjusted according to the patient’s body weight.

Non-emergency use of FXIII-containing products other than rFXIII was not allowed during the trial. In cases of acute bleeds, the investigator judged whether to treat with an additional FXIII-containing product in accordance with local standard practice. Additional rFXIII doses could not be used to treat any breakthrough bleeds.

For safety monitoring purposes, an additional interim visit (visit 3) was conducted 2 weeks after the first
dose of trial product. The follow-up period of 4 weeks after the last administration of trial product ensured that the majority of rFXIII would have cleared when patients ended their participation in the trial.

Patients ≥6 years of age, weighing ≥20 kg, with diagnosed congenital FXIII-A subunit deficiency (confirmed by genotype analysis) were enrolled. Patients who had received regular replacement therapy before entering the trial had to have initiated this treatment ≥6 months before screening, and have a documented history of ≥1 treatment-requiring bleed before starting regular replacement therapy or a documented family history of congenital FXIII deficiency. Patients who had only received on-demand treatment before entering the trial required a documented history of ≥2 treatment-requiring bleeds within 12 months before screening.

The rarity of congenital FXIII deficiency and low bleeding frequency of patients currently on regular replacement therapy did not allow for a sample size large enough for sufficient statistical power to compare clinical outcome between rFXIII and any other type of regular replacement. Furthermore, a placebo-controlled trial in congenital FXIII deficiency would have been unethical due to the risk of serious bleeding complications. The current trial was designed as a superiority trial: the primary efficacy parameter was the annualized frequency of bleeding events requiring treatment with FXIII-containing products during the rFXIII prophylaxis treatment period versus the historical bleeding rate in patients with congenital FXIII deficiency treated on-demand.

To define the historical control, Novo Nordisk conducted a comprehensive global survey among physicians, comprising 92 patients with congenital deficiency and asking how many bleeds per year patients had experienced. Of these, 23 patients were only treated on-demand to manage acute bleeds. Bleeding frequency data were not available for 4 patients receiving on-demand treatment, and in 3 patients, the diagnosis was made less than 1 year prior to the conduct of the survey. As a result, the statistical analysis was based on the remaining 16 patients, including 13 males and 3 females. Four of the
16 patients (25%) had no bleed requiring on-demand treatment, whereas 12 patients (75%) experienced bleeds requiring treatment. The number of bleeds requiring on-demand treatment ranged from 0 to 12 per year (mean 2.91 episodes per year). Based on a normal distribution approximation to the bleeding rate, a 95% confidence interval (CI) for the bleeding rate was calculated to be 0.95; 4.86.

Of the 92 patients in the survey, 69 had a history of regular FXIII replacement therapy. For 5 patients on prophylaxis, complete information regarding breakthrough bleeds was not available and data from these patients have, therefore, not been included in the calculations. A total of 17 of the 64 patients (26.6%) were reported to have experienced breakthrough bleeds while receiving regular replacement therapy. There were 20 breakthrough bleeds in total, ranging from 0 to 7 per year (data from 13 patients as the number of bleeds per year was not available for 4 patients with breakthrough bleeds). The annual bleeding rate estimated from 60 patients receiving prophylaxis was 0.33 bleeds per year (95% CI: 0.08; 0.59). Basic demographic data for patients receiving on-demand and prophylaxis treatment are summarized in Table 1. The mean age in the 2 populations was 31.7 and 28.6 years, respectively, which is comparable with the mean age of the current trial population.

**Patient genotype**

FXIII-A subunit deficiency was confirmed for all patients by genotyping. One patient also had a heterozygous missense mutation in the F13B gene, the effect of which is unknown. However, ELISA results for FXIII-B subunits showed that this patient had quantitatively normal B subunits which were functionally capable of binding to A subunits (as reflected by reduced levels of B subunits and increased FXIII-A₂B₂ levels following rFXIII injection).

**Patient assessments**

At each participating center, the local investigator collected a medical history and performed a physical
examination of each patient at baseline and at each subsequent study visit. Adverse events were recorded at every visit. Basic laboratory hematology, biochemistry, urinalysis and coagulation-related parameters (prothrombin time reported as international normalized ratio, activated partial thromboplastin time, thrombin time, and fibrinogen) were assessed at baseline and every visit. Additionally, the following tests were performed at baseline and every study visit at a central laboratory.

**FXIII activity assay**

A modified Berichrom® FXIII assay kit (Siemens Healthcare Diagnostics) was used to determine FXIII activity. The modifications refer to the use of rFXIII calibrators and buffer used for sample dilution. Overall assay precision (%CV) was 5-10% and accuracy (% relative error) was 4.3-10%. Recovery of FXIII activity was determined from the FXIII activity levels 1 hour after dosing and expressed as a percentage increase from the pre-dosing levels per unit of rFXIII administered per kilogram body weight (%/IU/kg).

**Clot solubility assay**

The clot solubility test is a qualitative assay to assess the clot strength. Plasma was allowed to clot following the addition of calcium. Then, 1% chloroacetic acid was added to the clot, which will dissolve within 24 hours if <1% FXIII is present. A normal result is greater than 24 hours without clot lysis. This assay is traditionally used as a primary screening test for the detection of FXIII deficiency; however, this assay only detects severe deficiencies, is poorly standardized, and varies in its degree of sensitivity.6,8-10 Similarly, the usefulness of the assay was not confirmed in the present study.

**FXIII-A₂ determination**

FXIII-A₂ was detected via an ELISA, using a polyclonal rabbit anti-FXIII-rhuA₂ subunit antibody (Novo Nordisk) to capture the FXIII-A₂ subunit. After incubation with plasma, bound FXIII-A₂ subunit was detected by incubation with biotin labeled polyclonal rabbit anti-FXIII-rhuA₂ subunit antibody and
streptavidin labeled with horse radish peroxidase (HRP). An Orto-Phenylendiamine hydrochloride (OPD) substrate solution was used for color development, which was directly proportional to the FXIII-A2 subunit concentration. Data were collected using a Versamax™ plate reader (Molecular Devices) and SoftMax® Pro GxP 5.0.1 software (Molecular Devices). The assays, performed in duplicates, were within inter-assay precision (%CV) and accuracy (% relative error) of <15%. The FXIII-A2 subunit assay measures total A2: FXIII-rhuA2 subunit, human endogenous FXIII-A2 subunit, human endogenous A2B2 tetramer, and FXIII-rhuA2 subunit bound to human endogenous B2 subunit (rhuA2B2 tetramer). rhu-FXIII was used to prepare the assay calibrators.

**FXIII-A2B2 determination**

FXIII-A2B2 was also detected using an ELISA, in which a polyclonal rabbit anti-FXIII-B subunit antibody (Novo Nordisk) was used to capture the FXIII-B subunit. After incubation with plasma, bound FXIII-A2B2 was detected by incubation with biotin labeled polyclonal rabbit anti-FXIII-A subunit antibody (Novo Nordisk) and subsequent incubation with streptavidin-HRP. Color development, using an OPD substrate solution, was directly proportional to the FXIII-A2B2 concentration. Data were collected as for the FXIII-A2 assay. The assay recognized rhuA2 subunit complexed to human B2 subunit (rhuA2B2), as well as endogenous human A2B2. Purified rhuA2B2 was used to prepare assay calibrators.

**Free FXIII-B determination**

Free FXIII-B subunit was determined by an ELISA, which used a polyclonal donkey anti-mouse IgG antibody (Jackson ImmunoResearch) as a first capture antibody to optimize binding of a second specific capture antibody, a monoclonal free B subunit antibody (Novo Nordisk). After incubation with plasma, free FXIII-B subunit was detected by incubation with a biotinylated polyclonal rabbit anti-FXIII-B subunit antibody (Novo Nordisk) and finally incubation with Streptavidin-HRP. A Tetra Methyl-Benzidine (TMB) substrate solution was used for color development, which was directly proportional to the free FXIII-B subunit concentration. Purified plasma FXIII-B subunit was used to prepare the
Detection of anti-rFXIII antibodies

All patients receiving rFXIII were monitored for the development of binding antibodies before administration of trial product at visits 1-16, as well as at any unscheduled visit.

**Step 1.** A direct ELISA was developed and validated to detect, confirm specificity, and quasi-quantify human immunoglobulin (Ig) against rFXIII. The rFXIII (10 μg/mL) was used as the coating agent and residual binding was blocked by 5% skimmed milk. Samples (ethylenediaminetetraacetic acid plasma, diluted 1:100) were applied and bound antibodies were detected using a secondary reagent (HRP conjugated polyclonal rabbit anti-human IgA, IgG, IgM, and kappa; DAKO). Following addition of TMB, substrate detection of antibodies was performed by reading the absorbance at 450 nm (reference 630 nm). An assay-plate-specific cut point was established using plasma from 100 healthy subjects and a normalization factor derived thereof (5% false positive rate). Samples above the cut point were rescreened and antibody specificity confirmed through pre-incubation with rFXIII (or unrelated protein [FVII]). Anti-rFXIII monkey plasma (Novo Nordisk) was used to establish assay quality controls. Samples containing antibodies against rFXIII were diluted 2-fold and results were reported as log(10) of the dilution needed to reach the cut point.

**Step 2.** Detection of the neutralizing activity of anti-rFXIII antibodies was based on the Berichrom® activity assay (Dade-Behring, Germany), but developed to detect anti-rFXIII antibodies’ neutralizing activity in clinical samples. The residual FXIII activity in a test sample was compared with a reference sample (pre-dose) after pre-incubation with a known amount of rFXIII activity (0.7 units/mL). The inhibitory effect in a study sample is expressed as 100% activity of study sample/activity of reference sample. The assay cut point (12% neutralization) was established during assay validation based on 5 FXIII activity-deficient plasma preparations. Quality controls were established using a sheep polyclonal
anti-FXIII-A_2 subunit antibody (SAF13A-IG, Affinity Biologicals).

**D-dimer**

D-dimer was measured using the Diagnostica Stago Asserachrom D-Dimer kit according to the manufacturer’s instructions.

**Statistical methodology**

The primary endpoint of the trial was evaluated by Poisson regression of the number of treatment-requiring bleeds, adjusting for baseline age as a covariate and for overdispersion. The total observation time during the treatment period was further used as an offset in the model, taking into account patients withdrawing before the end of the trial by adjusting for the length of time observed. An age-adjusted annualized bleeding rate was estimated, as well as a corresponding 95% CI. Superior efficacy of prophylaxis treatment with rFXIII was claimed if the upper 95% confidence limit was lower than the historic rate of 2.91 treatment-requiring bleeds/year in patients receiving on-demand treatment. As a stricter post-hoc comparison, the upper 95% confidence limit was compared with the lower 95% confidence limit (0.95) of the historic rate of treatment-requiring bleeds/year, based on a normal distribution approximation to the individual bleeding rate.

As a secondary endpoint, the percentage of patients without “treatment-requiring bleeds” with any FXIII-containing products was evaluated by a binomial model including age as a covariate to compare the data to a fixed placebo probability of whether or not patients experienced any treatment-requiring bleeds. Using historical data for patients receiving on-demand treatment, the probability of not having any treatment-requiring bleeds was estimated by a binomial model to be 0.25 (95% CI: 0.10; 0.51). Monthly replacement therapy with rFXIII would be concluded to be superior to on-demand treatment with FXIII-containing products if the lower limit of the 95% CI for the probability of not having any treatment-requiring bleeds for the rFXIII group, \( P \), was \( \geq .25 \).
Additional secondary descriptive endpoints comprised: (i) Annualized treatment-requiring bleeds stratified according to age, type, and cause of bleeding episodes, (ii) Percentage of patients having a normal clot solubility 1 hour and 28 days after rFXIII administration, (iii) Number of patients withdrawn from the trial due to lack of efficacy of rFXIII treatment, and (iv) Level of FXIII activity 1 hour and 28 days after rFXIII administration.

Results

Patients

Forty-one patients were enrolled and dosed (mean age: 26.4 years, range: 7-60 years; 56% male; 68% Caucasian; Table 2), with a mean treatment period of 322 days. All patients, except 2, received regular replacement therapy with FXIII-containing products prior to study enrolment. Five patients withdrew from the trial. Three discontinued rFXIII treatment due to non-neutralizing antibodies and returned to the previous local standard of treatment; however, they remained in the trial for follow up and completed other trial-related activity visits. The remaining 33 patients completed the predefined trial treatment period (Table 2).

Bleeding episodes

During the rFXIII treatment period, 5 bleeding episodes treated with a FXIII-containing product were observed in 4 patients, resulting in a mean annualized bleed rate of 0.138 bleeds per patient/year. All 5 events were traumatic bleeds (Table 3). No intracranial hemorrhage or severe bleeds into internal organs occurred during rFXIII treatment. Bleeds occurred on days 5, 14, 15, 22, and 27 post-dose with rFXIII. There is no statistical evidence to conclude that treatment-requiring bleeds occurred more frequently in the late post-dose phase, when plasma levels of rFXIII are expected to be lower.
In the primary endpoint analysis, the age-adjusted rate (number per patient year) of treatment-requiring bleeds during the rFXIII treatment period was 0.048/year (95% CI: 0.0094; 0.2501; model-based estimate corresponding to mean age of the trial population of 26.4 years). The influence of age was statistically significant with a $P$-value of 0.022.

Compared with retrospectively collected data from patients with congenital FXIII deficiency, the bleeding frequency in the present trial was numerically lower than for patients on regular replacement therapy (on average approximately 0.33 treatment-requiring bleeds/year). It was also significantly lower than the rate of treatment-requiring bleeds/year in patients receiving on-demand treatment, both when comparing with the historic rate (2.91 bleeds/year), and the lower 95% confidence limit (0.95 bleeds/year).

Thirty-seven of the 41 patients did not experience any bleeds requiring treatment during the trial. Age-adjusted statistical analysis via a binomial model determined the probability of not having any treatment-requiring bleeds during the trial period to be 0.9581/year (95% CI: 0.7242; 0.9950). No patients withdrew due to lack of efficacy of rFXIII treatment. During the rFXIII treatment period, 48 nontreatment-requiring bleeds were observed. The frequency of minor (nontreatment requiring) bleeds had similar distribution within all age groups with the exception of an 8-year-old patient who experienced 18 minor bleeds (mostly trauma induced).

**FXIII laboratory parameters**

**FXIII subunits and tetramer analyses.** The shape of the mean profiles for FXIII-A$_2$B$_2$ tetramer and total FXIII-A$_2$ subunit corresponded to the FXIII activity profile. FXIII-A$_2$ subunit and FXIII-A$_2$B$_2$ tetramer concentrations increased 4.5- and 7.6-fold, respectively, after rFXIII administration (Figure 1), then gradually declined during the subsequent month. The mean profile for uncomplexed B subunit concentration was reversed (Figure 1) and decreased 4-fold after rFXIII injection. This was expected, as the FXIII-B subunit functions as a carrier protein for the FXIII-A$_2$ subunit. As such, in the absence of
FXIII-A$_2$ subunit, there would be an excess of free FXIII-B subunit, whereas administration of rFXIII-A$_2$ subunit results in rapid binding to free FXIII-B subunits and subsequently decreases the concentration of free FXIII-B subunits.

**FXIII activity.** Mean pre-dose trough levels of FXIII activity (corresponding to 4 weeks after the preceding dose of rFXIII) were 0.19±0.05 IU/mL (Figure 2). As expected, rFXIII significantly elevated the mean FXIII Berichrom® activity at 1 hour post-dose to 0.77±0.20 IU/mL (both trough and 1 hour post-dose statistics as calculated over mean activity level per patient). The overall recovery of FXIII activity at 1 hour post-dose was similarly estimated to be 1.68±0.51 (IU/mL)/(IU/kg).

As a post-hoc analysis, the half-life of FXIII activity was estimated by a single exponential model for mean FXIII activity levels—calculated over all records—at 3 time points (1 hour: 0.78 IU/mL; 14 days: 0.30 IU/mL; 28 days: 0.19 IU/mL) to be approximately 11.8 days. This is consistent with the calculated half-life from the phase 1 trial.7

**Clot solubility test.** The occurrence of a positive clot lysis assay did not appear to be associated with the onset of treatment-requiring bleeds or temporal trends in the pre- or post-dose FXIII activity for the individual patient. However, positive clot lysis seemed to occur sporadically throughout the trial period and did not show a consistent trend in any of the investigated patients. Therefore, these data are not presented.

**Safety assessment**

In total, 231 adverse events occurring after initiation of rFXIII administration were reported in 32 patients. The most commonly reported events were headache (21 events in 12 patients), incorrect dosing (14 events in 7 patients), nasopharyngitis (11 events in 8 patients), and pyrexia (7 events in 7 patients). The vast majority of events were otherwise of mild to moderate severity. Eight serious adverse events
were observed in 6 patients (Table 4), all of whom recovered completely from their event(s). The remaining 170 events were not serious and had a frequency of less than 3%.

One patient was withdrawn from the trial by the investigator due to worsening of previously existing leucopenia and neutropenia. The patient had mild neutropenia (neutrophil count: 1200/μL; normal range: 2500-7500/μL) before the initial trial drug administration. The neutrophil count dropped to 940/μL at week 12, at which point the patient was withdrawn from the trial. The neutrophil count at the end-of-trial visit (week 16) was low at 1350/μL, but returned to pretreatment value.

Overall, no significant changes were observed over time in hematology and biochemistry parameters or fibrinogen, prothrombin time, activated partial thromboplastin time, D-dimer, and thrombin time. No thromboembolic events or deaths were reported.

Four patients (aged 8, 8, 14, and 16 years; including 2 siblings) developed transient, low-titer (2.3-2.6; lowest quantification level was 2.0 in logarithmic scale), non-neutralizing anti-rFXIII antibodies after starting rFXIII treatment. No neutralizing activity was identified by functional (inhibitory) assay at any time point. Two siblings (14-year-old male and 16-year-old female) developed antibodies after the first exposure to rFXIII. Treatment with trial product was discontinued as non-neutralizing antibodies had not previously been described and the risk of inhibitor development or changes in the pharmacokinetics were unknown. These patients resumed their local standard treatment but continued to be monitored throughout the trial. The antibody titer declined below the detection limit at 4 and 8 months after initial rFXIII treatment for the 14-year-old and 16-year-old patient, respectively. The third patient, an 8-year-old male, also developed antibodies after the first exposure to rFXIII. Despite the non-neutralizing antibodies decreasing below the level of detection at the time of the second dose, the patient’s parents withdrew informed consent and rFXIII was discontinued. The fourth case was an 8-year-old male who developed
non-neutralizing antibodies after the second exposure to rFXIII. He continued receiving monthly
treatment with rFXIII, and the antibody titer declined below the detection limit 4 months later.

No allergic reactions, treatment-requiring bleeds, or changes in pharmacokinetics were observed in any of
these patients at any time while the non-neutralizing antibodies were present or during follow-up.
Furthermore, the antibodies declined below the detection limit in all patients despite repeated exposure to
any FXIII-containing products: in 2 of the patients while receiving rFXIII, and in the remaining 2 patients
while receiving other FXIII-containing products

Discussion
Congenital FXIII deficiency is a severe bleeding disorder often manifesting at an early age with
intracranial bleeding. Safe, reliable, and effective therapy for this condition is highly warranted.
Previously, only plasma-derived sources of FXIII existed, which have an associated low risk of blood-
borne infections and allergic reactions. Utilization of advanced recombinant DNA technology can benefit
FXIII production by generating a uniform and highly purified rFXIII product containing only FXIII-A
subunit (identical to endogenous human FXIII). This product is manufactured without using animal or
human proteins, thus eliminating the risk of viral transmission. In addition, rFXIII production is
independent of donor plasma availability, and enables a more convenient administration with a smaller
volume.

This multicenter, multinational, open-label trial was undertaken to evaluate the efficacy and safety of this
novel rFXIII in patients with congenital FXIII deficiency. The results confirm that prophylaxis with
rFXIII is safe and effective for preventing bleeds in patients with congenital FXIII-A subunit deficiency.

Following injection of rFXIII, a substantial increase in FXIII-A_{2} subunit, FXIII-A_{2}B_{2}, and FXIII activity
was observed. The estimated half-lives of FXIII-A₂ subunit, FXIII-A₂B₂, and FXIII activity were similar to those reported for plasma derived FXIII-containing products,¹¹ and for rFXIII in the previous phase 1 study.⁷

The rFXIII was well tolerated. During the rFXIII treatment period, treatment-requiring bleeds were observed in only 4 out of 41 participating patients. In all cases these bleeds were associated with trauma and included nose and lip bleeds, soft tissue bleeds around the elbows, and facial bruising. Although the study protocol requested the assessment of FXIII activity prior to treatment of bleeds with any FXIII-containing product, blood samples were not collected prior to any of these events due to urgent medical attention for these cases. The age of patients who suffered bleeds requiring treatment was 8, 10, 16, and 19 years. Considering that the mean age of the trial population was 26 years, there was a tendency for treatment-requiring bleeds to occur primarily in younger patients, consistent with the effect of age being statistically significant in the primary analysis (P = .022). However, it should be kept in mind that all treatment-requiring bleeds were due to trauma, and the underlying reason for the apparent age affect is not known. We suspect that it might be because children and adolescents suffer trauma more frequently due to their level of physical activity.

No spontaneous treatment-requiring bleeds or intracranial hemorrhage occurred during the rFXIII treatment period. When compared with retrospectively collected data from patients with congenital FXIII deficiency, the bleeding frequency in the present trial was significantly lower than the rate of 2.91 treatment-requiring bleeds per year in patients receiving on-demand treatment. It should be noted that surveyed patients receiving on-demand treatment are likely to have a less severe disease state and perhaps bleed less often compared with prophylaxis patients prior to starting regular prophylaxis. The outcome of this survey study is similar to other data sources: for example, in a small study of 7 patients, the mean annual number of spontaneous bleeds was 2.5 events per year prior to Fibrogammin® P prophylaxis and 0.2 events per year during Fibrogammin® P prophylaxis.¹² Yoshida et al reported that bleeds markedly
decreased from 4.2±1.5/year to 0.2±0.2/year with no life-threatening hemorrhage, including intracerebral hemorrhage, in 4 patients given regular replacement therapy with Fibrogammin® P every 4 weeks for 10-19 years. Finally, a recent prospective study showed that, under prophylaxis with Fibrogammin® P, the majority of patients with FXIII deficiency had no hemorrhage, supporting the effectiveness of prophylactic treatment.

The most feared complication when introducing new factor concentrates is inhibitor development. In contrast to hemophilia A where cumulative incidence of inhibitor development ranges from 15 to 30%, the incidence of inhibitory antibodies in patients with congenital FXIII deficiency is very rare and has been reported in only 5 cases treated with plasma-based products. The development of an inhibitor in hemophilia or congenital FXIII deficiency substantially complicates continued management of the patient. Thus, this trial closely evaluated and monitored for antibody development to FXIII by testing patients monthly for anti-rFXIII antibodies. Importantly, no inhibitory antibodies were found in this trial.

Four of 41 patients developed transient, low-titer, non-neutralizing anti-rFXIII antibodies. These antibodies did not inhibit FXIII activity and patients continued to be treated with either rFXIII or plasma-derived FXIII. The anti-rFXIII antibodies were of the IgM isotype in 3 of the 4 patients, with no increase in antibody levels or isotype switching. Analysis of antibody isotype in the fourth patient was inconclusive due to antibody levels being too low to allow characterization. The presence of these non-neutralizing antibodies was not associated with any treatment-requiring bleeds, changes in FXIII pharmacokinetics, or allergic reactions. Furthermore, the antibodies declined below the detection limit in all patients, despite repeated exposure to rFXIII or other FXIII-containing products. These data indicate that the observed low titer, specific, non-neutralizing antibodies were not clinically significant. The 4 patients who developed these noninhibitory antibodies were all young adolescents (ages 8-16) who had
received prophylaxis with FXIII concentrates for many years before this trial. It is possible that patients with FXIII deficiency, when exposed to plasma-derived sources of FXIII, might also develop transient non-neutralizing antibodies, but this has not been evaluated in previous studies. Development of non-neutralizing antibodies is known to occur in patients with hemophilia and in normal healthy individuals with no underlying bleeding disorders. Although the 4 events of non-neutralizing, low-titer antibodies all occurred in patients below the age of 18 years, it is not known why antibodies have been detected more frequently upon initiation of treatment in children and adolescence. The present study did not suggest a relationship between genetic mutations and a predisposition for antibody development in these patients or any indication of inhibitor development; however, long-term follow-up studies are highly warranted to understand the immunogenic reaction in this rare bleeding disorder.

Apart from the issue of antibody development, there were no other safety issues and rFXIII was well tolerated. No thromboembolic or fatal adverse events were reported.

The small numbers included in this trial may be a limiting factor of this study and conclusions observed. However congenital FXIII deficiency is a rare disease and the trial included 41 patients, accounting for approximately 7% of the diagnosed congenital FXIII deficiency population worldwide. To date, this is the largest completed prospective clinical trial in patients with congenital FXIII deficiency. The present study demonstrated that rFXIII as monthly replacement therapy is efficacious and safe for prophylactic treatment in patients with congenital FXIII-A subunit deficiency.
Acknowledgements

This trial was supported by Novo Nordisk A/S. We would like to thank all the patients who participated in the trial and the physicians who enrolled them including Drs Carmen Altisent, Brigitte Brand, Giancarlo Castaman, Marie Dreyfus, Wolfgan Eberl, Susan Halimeh, John Hanley, Neil Josephson, Christine Kempton, Gili Kenet, Bryce Kerlin, Riitta Lassila, Ri Liesner, Ellis Neufeld, Roland Riess, Kris Ann Schultz, Charles Sexauer, Cameron Tebbi, and Henry Watson. We thank Dr Yuen-Sum Man (employed by Novo Nordisk) for her critical review of the manuscript. Editorial assistance to the authors during the preparation of this manuscript was provided by Sharon Eastwood (medical writer, PAREXEL) and financially supported by Novo Nordisk A/S in compliance with international guidelines for good publication practice.

Authorship and conflicts of interest

Authorship

I.A. conducted the trial, reviewed the data and wrote the manuscript. J.O. contributed to the research, data collection and analysis, conception, and reviewing of the manuscript. M.C. performed the research, data collection and analysis, and wrote the manuscript. A.R. analyzed the data, reviewed, and contributed to writing the manuscript. R.T. contributed to the design of the study, performed the research, collected and analyzed the data, and wrote the manuscript. D.N. contributed to the design of the study, performed the research, analyzed the data, and wrote the manuscript.

Conflicts of interest statements

I.A. has no conflicts of interest to declare. J.O. received reimbursement for attending symposia/congresses and/or honoraria for speaking and/or honoraria for consulting, and/or funds for research from Baxter, Bayer, Biogen Idec, Biotest, CSL Behring, Grifols, Inspiration, Novo Nordisk, Octapharma, Pfizer, and
Swedish Orphan Biovitrum. M.C. received grant support from Bayer, Baxter, CSL, Novo Nordisk, and Pfizer, and honoraria for speaking at events sponsored by Bayer, Baxter, CSL Behring, Grifols, Novo Nordisk, and Pfizer. A.R. and R.T. are Novo Nordisk employees and hold Novo Nordisk stock options. D.N. received honoraria for speaking at events sponsored by Bayer, Baxter, CSL Behring, Grifols, Novo Nordisk, and Pfizer.
References


### Table 1. Demographic data at the time of data collection

<table>
<thead>
<tr>
<th></th>
<th>On-demand</th>
<th>Prophylaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>16</td>
<td>60</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, SD</td>
<td>31.7 (17.6)</td>
<td>28.6 (16.1)</td>
</tr>
<tr>
<td>Median</td>
<td>32.0</td>
<td>28.0</td>
</tr>
<tr>
<td>Min; Max</td>
<td>5.0; 63.0</td>
<td>2.8; 71.0</td>
</tr>
<tr>
<td>Gender, N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>3 (19)</td>
<td>36 (60)</td>
</tr>
<tr>
<td>Male</td>
<td>13 (81)</td>
<td>24 (40)</td>
</tr>
<tr>
<td>Race, N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>11 (69)</td>
<td>55 (92)</td>
</tr>
<tr>
<td>Asian</td>
<td>0 (0)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Black or African American</td>
<td>3 (19)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (12)</td>
<td>5 (3)</td>
</tr>
</tbody>
</table>
**Table 2. Patient disposition and baseline demographics**

<table>
<thead>
<tr>
<th>Patient disposition, N</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrolled and dosed</td>
<td>41</td>
</tr>
<tr>
<td>Withdrawals</td>
<td>5</td>
</tr>
<tr>
<td>Inconvenience (parent’s decision; visit 5)</td>
<td>1</td>
</tr>
<tr>
<td>Adverse events of worsening leukopenia and neutropenia (visit 6)</td>
<td>1</td>
</tr>
<tr>
<td>Pregnancy (visit 8 and 14)*</td>
<td>2</td>
</tr>
<tr>
<td>Personal reasons (visit 9)</td>
<td>1</td>
</tr>
<tr>
<td>Discontinuation of rFXIII treatment due to detection of non-neutralizing antibodies</td>
<td>3†</td>
</tr>
<tr>
<td>Completed trial</td>
<td>33</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Baseline demographics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>41</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
</tr>
<tr>
<td>Mean, SD</td>
<td>26.4 (15.9)</td>
</tr>
<tr>
<td>Median</td>
<td>23.0</td>
</tr>
<tr>
<td>Min; Max</td>
<td>7.0; 60.0</td>
</tr>
<tr>
<td>Gender, N (%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>18 (44)</td>
</tr>
<tr>
<td>Male</td>
<td>23 (56)</td>
</tr>
<tr>
<td>Race, N (%)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>28 (68)</td>
</tr>
<tr>
<td>Asian</td>
<td>5 (12)</td>
</tr>
<tr>
<td>Black or African American</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (12)</td>
</tr>
<tr>
<td>Unknown‡</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>
* One pregnancy was identified through pregnancy screening and the other was reported by the investigator.

† Of the 3 patients who discontinued rFXIII treatment due to the detection of non-neutralizing antibodies, the decision was made by the Novo Nordisk Safety Committee for 2 patients (following recommendations from contracted external experts who reviewed data from these patients) and by the patient’s parents for 1 patient. All 3 patients remained in the trial for follow-up and completed all trial-related activity visits.

‡ Not permitted by local authority.
### Table 3. Details of bleeding episodes requiring treatment

<table>
<thead>
<tr>
<th>Gender, Age (years)</th>
<th>Days since last dose</th>
<th>Bleeding location</th>
</tr>
</thead>
<tbody>
<tr>
<td>F, 8</td>
<td>15</td>
<td>Lip</td>
</tr>
<tr>
<td>M, 10</td>
<td>22</td>
<td>Soft tissue around elbow</td>
</tr>
<tr>
<td>M, 10</td>
<td>14</td>
<td>Soft tissue around elbow</td>
</tr>
<tr>
<td>M, 16</td>
<td>5</td>
<td>Nose</td>
</tr>
<tr>
<td>F, 19</td>
<td>27</td>
<td>Bruises to nose and face</td>
</tr>
</tbody>
</table>

F indicates female; M indicates male.
Table 4. Serious adverse events

<table>
<thead>
<tr>
<th>Patient age (years)</th>
<th>Preferred term</th>
<th>Days from dosing to onset</th>
<th>Relation to trial drug*</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Antibody test positive</td>
<td>15</td>
<td>Probable</td>
<td>Recovered</td>
</tr>
<tr>
<td></td>
<td>Antibody test positive</td>
<td>17</td>
<td>Possible</td>
<td>Recovered</td>
</tr>
<tr>
<td>14</td>
<td>Antibody test positive</td>
<td>4</td>
<td>Unlikely</td>
<td>Recovered</td>
</tr>
<tr>
<td>16</td>
<td>Antibody test positive</td>
<td>17</td>
<td>Possible</td>
<td>Recovered</td>
</tr>
<tr>
<td>19</td>
<td>Road traffic accident</td>
<td>28</td>
<td>Unlikely</td>
<td>Recovered</td>
</tr>
<tr>
<td>55</td>
<td>Noncardiac chest pain</td>
<td>23</td>
<td>Unlikely</td>
<td>Recovered</td>
</tr>
<tr>
<td>57</td>
<td>Diverticulitis</td>
<td>3</td>
<td>Unlikely</td>
<td>Recovered</td>
</tr>
</tbody>
</table>

*Relationship to Trial Product Assessment Definitions:

• Probable: Good reasons and sufficient documentation to assume a causal relationship
• Possible: A causal relationship is conceivable and cannot be dismissed
• Unlikely: The event is most likely related to an etiology other than the trial product.
Figure legends

Figure 1. Mean±SD concentration of FXIII-A$_2$ subunit (A) A$_2$B$_2$ tetramer (B), and B subunit (C).

FXIII-A$_2$ subunit and FXIII-A$_2$B$_2$ tetramer concentrations increased following rFXIII administration. As the FXIII-B subunit functions as a carrier protein for the FXIII-A$_2$ subunit, administration of rFXIII resulted in decreased FXIII-B subunit due to the rapid binding of rFXIII to free FXIII-B subunit.

Figure 2. Mean profile of FXIII Berichrom$^\text{®}$ activity (IU/mL) shown with standard error of the mean per visit. Dosing with rFXIII resulted in the maintenance of average FXIII activity trough levels above 0.10 IU/mL throughout the rFXIII prophylaxis period.
Recombinant factor XIII: a safe, and novel treatment for congenital factor XIII deficiency

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